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<p>(54) Title: METHOD OF ASSAYING FOR DRUGS WHICH RESTORE FUNCTION OF MUTANT CFTR PROTEIN</p> <p>(57) Abstract</p> <p>A method of identifying potentially useful treatments which restore the function of CFTR or other related protein mutations is disclosed. The method involves creation of yeast STE6-CFTR chimeric sequences which upon introduction of a desired mutation inhibit expression of the yeast STE6 gene. A yeast assay (STE6Δ) transformed to include the hybrid chimeras is then exposed to a potential treatment for the mutation. A treatment which restores the function of the STE6 gene and thereby allows mating of the yeast strain is indicative of a potential remedial treatment for the mutation in this selected protein.</p>		

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**Title:       METHOD OF ASSAYING FOR DRUGS WHICH RESTORE  
              FUNCTION OF MUTANT CFTR PROTEIN**

**BACKGROUND OF THE INVENTION**

Cystic fibrosis is a human genetic disease of the secretory epithelia. Although the survival rate of those suffering with cystic fibrosis has improved in recent years, the median age for patient survival is still only about twenty five to thirty years despite intensive supportive and prophylactic treatment. Today cystic fibrosis remains the most common congenital disease among Caucasians, where it has a prevalence of about one in two thousand live births, and is uniformly fatal. Nearly all patients suffering from the disease develop chronic progressive disease of the respiratory system, the most common cause of death being pulmonary disease. Also, in the majority of cases, pancreatic dysfunction occurs; hepatobiliary and genitourinary diseases are also frequent. Because of the multi-system clinical manifestations of the disease, current methods of treatment for the disease have focused on therapeutic approaches to reduce the symptoms of cystic fibrosis.

United States Patent No. 5,100,647 to Agus, et al, discloses a method for treating cystic fibrosis by administration of the compound sparteine (dodecahydro-7, 14methano-2H, 6H-di-pyrido [1,2-a: 1',2'-e] [1,5] diazocine), acting as a direct exogenous activator of chloride conductants in epithelial airways. United States Patent No. 5,179,001 to Young, et al, discloses a method of treating pulmonary complications associated with cystic fibrosis caused by the gram negative bacterium Pseudomonas aeruginosa. United States

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Patent No. 4,826,679 to Roy relates to an oral composition for alleviating digestive manifestation in persons afflicted with cystic fibrosis comprising a therapeutic amount of taurine. Despite much advancement in the treatment of the symptoms of cystic fibrosis very little has been done to effectively "cure" the disease at a genetic level.

One method of gene therapy proposed is United States Patent No. 5,149,797 disclosing a method of site specific alteration of RNA and production of encoded polypeptides. This invention is drawn to correcting the abnormal mRNA present in individual cells, cleaving the mRNA by site directed RNAase followed by introduction of the appropriate oligoribonucleotide followed by endogenous RNA ligase and thus production of a wild-type mRNA encoding a normal protein product which then may be translated to produce the correct protein.

Cystic fibrosis is characterized at the genetic level by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The CFTR protein is a member of a superfamily of structurally related membrane proteins named the traffic ATPase or ATP-binding cassette (ABC) transporters. Other members of this superfamily include the multi-drug resistance (MDR) or P-glycoprotein, bovine adenylyl cyclase, the yeast STE6 protein, as well as several bacterial amino acid transport proteins (Riordan, et al, 1989; Hyde, et al, 1990). The principle distinguishing feature of this superfamily of proteins is a highly conserved nucleotide-binding domain (NBD).

CFTR is a protein of approximately 1480 amino acids consisting of two repeated motifs, each

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comprising 6 transmembrane segments and a nucleotide-binding domain. The two motifs are separated by a large polar R-domain containing multiple potential phosphorylation sites.

Many members of the ABC transport superfamily transport small molecules across biological membranes in an ATP dependent manner. However, CFTR is a  $\text{Cl}^-$  channel regulated by phosphorylation and by cytosolic ATP (Anderson, et al, "Nucleoside Triphosphates are Required to Open CFTR Chloride Channel" *Cell*, 67, 775-784). The two nucleotide-binding domains present in CFTR play a central role in the function of CFTR  $\text{Cl}^-$  channels; they interact directly with ATP to open the CFTR  $\text{Cl}^-$  channel and ATP hydrolysis may be required for this effect. The importance of the NBD's is further emphasized by the large number of cystic fibrosis associated mutations that have been found in the domains. The most common cystic fibrosis associated mutation, accounting for approximately 68% of the cystic fibrosis chromosomes is deletion of a phenylalanine at position 508 ( $\Delta\text{F508}$ ) in the middle of NBD1.

This mutation is characterized by a loss of apical membrane  $\text{Cl}^-$  channel activity in the epithelial cells leading to chronic progressive disease of the respiratory system. The  $\Delta\text{F508}$  mutation causes loss of apical membrane  $\text{Cl}^-$  channel activity affecting the CFTR protein in two ways. First, the mutation causes defective processing and hence mislocalization of the mutant protein. This is presumed to occur from misfolding of the mutant protein so that it fails to exit from the endoplasmic reticulum and progress to the Golgi complex in the apical membrane. The resulting  $\text{Cl}^-$

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transport defect associated with the  $\Delta F508$  is thus largely a consequence of the absence of functional CFTR at the plasma membrane. Second the  $\Delta F508$  mutation affects the function of the  $Cl^-$  channel. Single channel analysis of CFTR  $\Delta F508$  suggests that although the mutant protein is functional, it has an open state probability ( $P_o$ ) that is one third that of wild-type CFTR.

Defective trafficking of CFTR  $\Delta F508$  can be partially reversed if cells are grown at a lower temperature, indicating that mutant protein is still functional. When CFTR  $\Delta F508$  is produced at a reduced temperature (less than  $37^\circ C$ ) some of the mutant protein transits the Golgi complex normally and is localized correctly in the plasma membrane. (See Denning, G.M.; Anderson, M.P.; Amara, J.; Marshall, J.; Smith, A.E.; and Welsh, M.J.); "Processing of Mutant CFTR ( $\Delta F508$ ) is Temperature Sensitive." *Nature*, 358, 761-764. Since CFTR  $\Delta F508$  retains partial function, a possible therapy for cystic fibrosis may involve pharmacologic intervention designed to correct the processing defects so that more CFTR  $\Delta F508$  reaches the plasma membrane. Potential pharmacologic intervention would represent a non gene-therapy approach for the treatment of cystic fibrosis.

As can be seen, a great need exists in the art for methods of treating cystic fibrosis that do not concentrate solely on the symptoms produced by the disease, but rather treat the disease at a cellular level by attempting to restore function to the mutant product. In light of the scientific potential for pharmacologic treatments, a method for identification of new candidate drugs for cystic

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fibrosis treatment is warranted. This is complicated, however, by the fact that a screening method to identify treatments that reverse the CFTR  $\Delta F508$   $Cl^-$  transport defect in mammalian cells is impractical; large numbers of chemical compounds cannot easily be screened for an activity that restores the cAMP-stimulated  $Cl^-$  transport of mutant CFTR.

It is an object of this invention to provide an in vitro method of screening pharmaceutical compositions, and further mutations for their ability to restore function to defective ABC transport proteins resulting from mutations such as CFTR  $\Delta F508$ , by simple means of a yeast mating assay.

It is another object of the invention to construct chimeric gene sequences which encode the yeast STE6 gene and the CFTR gene for screening of treatments which will restore function to the mutant proteins.

It is another object of the invention to construct chimeric gene sequences which encode the yeast STE6 gene and the P-glycoprotein gene (or other ABC transporter genes) for screening treatments which will inhibit the function of the chimeric protein.

Yet another object of the invention is to provide a method for identifying potentially useful compounds for further useful study in the treatment of disease by means of a yeast mating assay.

Other objects of the invention will become obvious from the description of the invention which follows.

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SUMMARY OF THE INVENTION

This invention relates to a method for screening treatments which restore the function of mutant CFTR by simple means of a yeast cell mating assay. According to the present invention, chimeric genetic sequences which encode the yeast  $\alpha$ -factor transporter STE6 and CFTR or another member of the related superfamily such as P-glycoprotein are constructed whereby a segment of the CFTR genetic sequence is exchanged for a corresponding segment of the STE6 genetic sequence to produce a product which will complement a yeast  $ste6\Delta$  mutation but is non-functional upon introduction of a desired mutation into the CFTR sequence.

The resulting chimeras are then transformed into a yeast strain which has the STE6 gene deleted ( $ste6\Delta$ ). The yeast strain is then exposed to the desired drug or treatment to be screened and the strain is assayed for the presence of restoration of mating, which indicates a functional chimera. The invention discloses several chimeric sequences useful for screening for utility of treatments and reversing the effects of the CFTR  $\Delta F508$  mutation which is associated with cystic fibrosis.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 represents the amino acid sequence alignment of NBD1 of STE6 and CFTR.

Figure 2 depicts the STE6-CFTR chimeric constructs and their mating efficiency.

Figure 3 is a graphical depiction of a yeast assay according to the present invention which presents a potential cystic fibrosis treatment.



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Figure 4a-g is the gene sequence for chimera H4.

Figure 5a-g is the gene sequence for chimera H5.

Figure 6a-g is the gene sequence for chimera H6.

#### DETAILED DESCRIPTION OF THE INVENTION

The STE6 gene of Saccharomyces cerevisiae encodes an integral membrane protein that functions to transport the peptide a-factor out of the cell during mating. (Kuchler and Thorner, 1992), "Functional expression of human MDR1 in yeast Saccharomyces cerevisiae." *Proc. Natl. Acad. Sci.*, USA89, 2303-2306. Deletion of the STE6 gene from yeast (ste6 $\Delta$ ) results in a sterile phenotype. According to the present invention, chimeric STE6 genes were constructed in which segments of the NBD1 of STE6 were substituted by the analogous NBD1 sequences from CFTR.

Selection for sites of exchange was accomplished by comparing the known amino acid sequences of CFTR and STE6. The CFTR and STE6 amino acid sequences were aligned by use of the "BEST FIT" algorithm for the comparison of DNA and amino acid sequences obtained from the University of Wisconsin and found in the GCG Sequence Analysis Package.

Figure 1 represents an amino acid sequence alignment of the NBD for proteins CFTR and STE6. The CFTR amino acid sequence is depicted on top illustrating amino acids 442 through 578; the corresponding analogous STE6 amino acid sequence is depicted below illustrating amino acids 376 through 536. Corresponding amino acids are indicated by

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solid vertical bars and give an indication of potential junctions for the genetic constructs.

Table 1 indicates the portion of CFTR amino acids sequence inserted into STE6 for the different hybrids.

TABLE 1

<u>HYBRID IDENTIFIER</u>	<u>PORTION OF CFTR AMINO ACID SEQUENCE</u>
H1	D443-Y577
H2	D443-F508
H3	F494-F508
H4	F494-I546
H5	F494-L558
H6	F494-Y577

The H1 STE6-CFTR chimeric gene was constructed with a DNA sequence coding for the entire NBD1 of STE6 (from N377 to A535) replaced by the corresponding region of CFTR (D443 to Y557). (See Figure 1.) H2 replaced the amino terminal half of NBD and H6 replaced the carboxy terminal half. The remaining constructs were variations between these two extremes. (See Figure 2.) The construction of plasmids with these sequences used methods which are well known to those of skill in the art however the following description is included merely for purposes of illustration and is not intended to limit in any way the invention.

#### PLASMID CONSTRUCTIONS

Plasmid RFG416 (a gift from Rick Gaber, Northwestern University) is a single copy CEN plasmid with the selectable marker URA3 and the pUC19 polylinker region. A 6.5 kb Sall-SacI fragment containing the STE6 gene was subcloned from STE6-2 $\mu$  (a gift from John McGrath, Massachusetts

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Institute of Technology) into the Sall and SacI sites of the vector RFG416 to produce plasmid JTS6. The yeast TRP1 gene (on a 0.85 kb BglII-EcoRI DNA fragment) was inserted at nucleotide position 1356 of STE6 NBD1, resulting in JTS6T.

To construct the H1 STE6-CFTR hybrid plasmid, a 441 bp DNA fragment containing the CFTR NBD1 region was synthesized using two STE6-CFTR oligonucleotide primers (primer 1, 5'CCTTCGGAAGCAGTCCTGAAAGATAT-3'; primer 2, 5'-GATGAACAATATCTAGGTATCCAAAA-3') and CFTR cDNA template DNA in a polymerase chain reaction (PCR) (Ho, et al., 1989). PCR reactions were performed with a Temp-Tronic thermal cycler (Barnstead/Thermolyne). Oligonucleotide primers 1 and 2 encoded fusion junctions of STE6 L375 to CFTR K442 and CFTR 1578 to STE6 D537, respectively, found in the H1 STE6-CFTR hybrid gene. The CFTR portion of each primer is underlined. STE6 DNA flanking NBD1 was added to each end of the 441 bp fragment by PCR, resulting in a 867 bp DNA fragment consisting of 419 bp of CFTR DNA (encoding CFTR amino acids K442-L578) that is flanked at the 5' end by 168 bp of STE6 DNA (encoding STE6 amino acids K319-L375) and 280 bp of STE6 DNA at the 3' end (encoding STE6 amino acids D537-G640). Plasmid construction of H1 was performed by cotransformation (Ito, et al, 1983) of yeast strain JPY201 with the 867 bp DNA fragment and 3 µg of plasmid JTS6T and selection of transformants on SD-URA (yeast nitrogen base supplemented with all amino acids except uracil). Homologous recombination between the STE6 DNA sequences at each end of the 867 bp DNA fragment with the STE6 gene on the plasmid results in the targeted integration (Orr-Weaver, et al, 1981) of the CFTR sequences into

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NBD1 of STE6 on plasmid JTS6T and the consequent loss of the TRP1 gene. Recombinants containing the desired STE6-CFTR hybrid gene were identified as trp auxotrophs at a frequency of about 1% among the transformants. Plasmid DNA was prepared from trp transformants and the structure of the STE6-CFTR gene was confirmed by DNA sequencing analysis. STE6-CFTR hybrid genes H2-H6 were similarly constructed using the following oligonucleotide primers for constructing the appropriate STE6CFTR junctions; STE6 Q440/CFTR F494, 5'-CACCGTCGTAGAACAGTTTTTCCTGGATTA-3'; CFTR G509/STE6 S457, 5'-CCGAATCTGTTGAACCAAAGATGATATTT-3'; CFTR G550/STE6 G509, 5'-TTGTTGTTGCCCGCCACTCAGTGTGATTC-3'; CFTR R560/STE6 A519, 5' ATCTCTGATGAATGCTCTTGCTAAA-GAAAT-3'.

The gene sequences for H4, H5, and H6 are included in Figures 4-6.

The resulting plasmids were then transformed into a yeast strain with the STE6 gene deleted (ste6 $\Delta$ ) to test for complementation by means of a yeast mating assay.

#### YEAST CELL MATING ASSAY

Several types of variations of assays may be used and are commonly known to those of skill in the art. The following description is not intended to limit in any way the invention.

The yeast strains JPY201 (MATa ste6 $\Delta$ :HIS3, gal2, ura3-52, lys2-801, trp1, leu2-3,112, his3  $\Delta$ 200) and 22-2D (MATa, ura352, leu2-3,112, trp1) were used for all mating experiments. JPY201 contains a STE6 deletion (including NBD1 and extending beyond the termination codon) and replacement with the

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yeast HIS3 gene (McGrath and Varshavsky, 1989). Quantitative mating assays were performed as in Trueheart, et al (1987).

Transformants of JPY201 containing each STE6-CFTR chimera were grown to log phase in 0.1% glucose SD-URA media. From each culture,  $3 \times 10^6$  cells were mixed with an equal number of 22-2D cells grown in YPD media and collected by filtration onto a Millipore filter, which was then placed upon a YPD plate for 4 hours at 30°C. Cells were resuspended, sonicated briefly, and plated from serial dilutions onto SD+LEU/TRP (yeast nitrogen base supplemented with leucine and tryptophan). Diploid colonies were counted after 3 days at 30°C. Control strains STE6 wild-type and *ste6Δ* consisted of JPY201 transformed with plasmids JTS6 and JTS6T, respectively. For qualitative petri dish mating assays, JPY201 transformants containing STE6-CFTR chimeras were grown as patches on SD-URA media and then replica printed to YPD media on a lawn of 22-2D cells. Following incubation at 30°C for 8 hr, the plate containing the mating cells was replica printed to SD-LEU/TRP and incubated 3 days at 30°C to allow growth of diploid colonies.

The complementation results are given in Figure 2. As can be seen yeast transformants containing the H1 sequence were unable to complement the *ste6Δ* mutation, indicating a nonfunctional STE6 chimera. Similarly, the H2 sequence replacing the amino half only was nonfunctional. See Figures 1 and 2. These results suggest that the amino terminal region of NBD1 from CFTR cannot substitute for that of STE6.

In contrast, the STE6-CFTR chimeras containing the central part of NBD1 from CFTR (H3 and H4, See

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Figures 1 and 2) maintained a-factor transport activity comparable with the wild-type STE6 yeast strain. This suggests that amino acid residues between the conserved Walker A and B motifs (Walker, et al, 1982) although different in the NBD1 of CFTR and STE6 provide a similar overall structure and function. When the chimeras included progressively larger segments of the carboxy-terminal region of NBD1 from CFTR (H5 and H6) mating efficiency was reduced. Yeast transformed with the hybrid gene H5 which contained the substitution of 74 residues of STE6 NBD1 (from R441 to I516) by that from CFTR (from F494 to L558) mated at 12% efficiency of wild-type STE6. Mating efficiency was further reduced in H6 to background levels. However, a low level of mating was observed when the H6 gene construct was expressed on a high copy number plasmid.

#### INTRODUCTION OF THE $\Delta$ F508 MUTATION

A previous study has suggested that missense mutations in STE6 that were analogous to CF-associated mutations in NBD1 of CFTR result in defective a-factor transport, however, single amino acid deletions analogous to  $\Delta$ F508 within the central region of STE6 NBD1 had no effect on the STE6 function. (Berkower and Michaelis, "Mutational Analysis of the Yeast a-factor Transporter STE6; a Member of the ATP Binding Cassette (ABC Protein Superfamily)," *EMBO J* 10, 3777-3785 (1991)).

Contrary to this finding, it was found that by incorporating CFTR into NBD1 of the STE6 gene and also including the  $\Delta$ F508 mutation within the CFTR segment, function of the STE6 chimeric gene was inhibited. The chimeric sequence constructs H3, H4,

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H5, and H6 which demonstrated complementation of the *ste6Δ* yeast strain were also used to assess the effect of the  $\Delta F508$  mutation. The  $\Delta F508$  mutation was introduced into H3, H4, H5, and H6 (resulting in H3- $\Delta F508$ , H4- $\Delta F508$ , H5- $\Delta F508$ , and H6- $\Delta F508$  respectively). Figure 2 shows the effect of the  $\Delta F508$  mutation on a-factor transport as assessed by the quantitative yeast cell mating assay.

As seen from the results in Figure 2, yeast transformed with the H3- $\Delta F508$  chimera mated with an efficiency equal to the H3 control (no F508 mutation). A decrease in mating efficiency (40%) was observed with the strain containing the H4- $\Delta F508$  chimera relative to the H4 control. (Sequence I.D. No. 1). The  $\Delta F508$  mutation lowered mating efficiency by 80 fold in the H5- $\Delta F508$  chimera as compared with H5 (Sequence I.D. No. 3). The H6- $\Delta F508$  chimera displayed a comparable decrease in mating as compared to H6 (Sequence I.D. No. 5) efficiency due to the  $\Delta F508$  mutation when expressed on a high copy number plasmid, (and relative to the H6 chimera gene also on high copy number plasmid vector). For use in accordance with the present invention, expression of the H6- $\Delta F508$  gene on a high copy number plasmid would be required.

Based on the foregoing junction analysis, the portion of CFTR most promising for substitution into STE6 include the amino acid identity locations between a portion of NBD1 from amino acid 444 up to amino acid 509 through 577. As earlier seen, the segment from 443 to 557 was inactive as was the portion from 443 to 508. Preferred ranges include from amino acid 494 to 577 including: sequence I.D. No. 1, CFTR amino acid F494-I546; Sequence I.D. No.

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3, F494-L558; Sequence I.D. No. 5 CFTR amino acid F494-Y577. Several areas of identity between the two aligned sequences as demonstrated in Figure 1 exist within this range of CFTR amino acid substitutions and will represent other chimeric sequences useful for the present invention.

YEAST MATING ASSAY TO IDENTIFY  
COMPOUNDS THAT CORRECT THE  $\Delta$ F508 DEFECT

In accordance with the present invention, a yeast strain transformed to contain the discussed chimeric sequences will be used in a yeast mating assay to identify compounds which will correct the affect of the mutation as indicated by an increase in mating. Such an assay may be widely varied by those of skill in the art. Generally, the transformed strain will be combined with a strain of the alpha mating type on a suspension of yeast cells spread on the surface of agar medium in the petri dish. These two strains would be unable to mate due to the  $\Delta$ F508 mutation.

A compound is then introduced to the medium. For example a plant screening method may be performed by placing a small disc of leaf material removed by means of a paper punch from a plant and placed on the surface of the agar media. Plant compounds will diffuse from the leaf and interact with the yeast strains.

Another method would be to use a filter paper which has been soaked in the desired chemical and then placed on the yeast suspension. Further, other desired mutations can be introduced into the CFTR region to attempt to identify a revertant mutation and a corresponding protein for further study. It is to be understood that any form of exposing the



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yeast strain to the desired treatment may be used including modifying external conditions such as temperature.

The mating plate is then incubated for 24 hours to allow yeast cell mating to occur. If a compound corrects the molecular defect associated with H5- $\Delta$ F508 transporter then a-factor is transported allowing mating between the two strains, resulting in diploid cells. The mating plate is then replica printed to a selective media plate to allow growth of diploid colonies.

For example strain A could be - MATa, STE6::HIS3, ura3-52, lys2, trp1-298, his3-200, leu2-3,112 <plasmid H5- $\Delta$ F508, URA3, CEN>; strain B - MAT $\alpha$ , ura3-52, trp1, leu2-3,112.

The selective media used would then be lacking lysine and uracil. Consequently strains A and B could not grow on this media (strain A has the lys2 mutation and strain B has the ura3-52 mutation; these mutations prevent them from going on media without lysine and uracil respectively), however diploids produced as a result of the fusion between strain A and B can grow (diploids contain the genomes of both haploids and consequently has the complementing URA3 and LYS2 wild-type allele).

Production of yeast diploid cells in the area surrounding the treatment disc would be a strong indicator of presence of compound(s) which correct the  $\Delta$ F508 defect. A number of yeast mating type assays may be developed easily by those of skill in the art and the foregoing description is not intended to limit in any way the invention.

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EXAMPLE 1REVERTANTS OF  $\Delta$ F508 MATING DEFECT IN YEAST  
(MUTATION IN R553-L558 REGION TO SUPPRESS  $\Delta$ F508 IN NBD1)

The R553-L558 amino acid region of CFTR is of great interest in CFTR and other trafficking ATPase's as it is directly adjacent to the conserved LSGGQ linker sequence of NBD1, postulated to function as a transducer of signals between the hydrophobic domains and the NBD's (Mimura, et al, 1991). It was hypothesized that a possible treatment to correct the F508 mutation could be interaction of the region of the NBD polypeptide containing F508, with the R553-L558 region. The only difference between the H5 chimera and the H4 chimera is 6 amino acids in the R553-L558 region. This region is also not present in the H3 chimera in which the  $\Delta$ F508 mutation had no effect. Possibly there is an interaction between the two regions of the NBD required for correct folding or function (or both) of the NBD. Thus the method of the present invention was used to test whether a mutation in the region R553-L558 might suppress the adverse effects of the  $\Delta$ F508 in NBD1.

To isolate revertants of the H5- $\Delta$ F508 mating defect, the H5- $\Delta$ F508 construct was mutagenized in the R553-L558 region in vitro by site-specific oligonucleotide mutagenesis (Ho, et al, "Site-Directed Mutagenesis by Overlap Extension Using the Polymerase Chain Reaction Gene" 77,51-59 (1989)). The mutagenesis method was designed to introduce random amino acid substitutions at single codon positions within the R553-L558 region of the H5- $\Delta$ F508 plasmid. The procedure allowed 20 possible substitutions at each amino acid position within

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R553-L558. The mutagenized DNA was transformed into yeast, and transformant colonies were analyzed by cell mating assays on petri dishes to identify colonies with a mating efficiency higher than the unmutagenized H5- $\Delta$ F508 control. Two colonies identified displayed higher efficiencies of mating within the first 20 mutant transformants analyzed. These yeast transformants each contained an H5- $\Delta$ F508 plasmid with a mutation at amino acid R553 of CFTR; in one case, R553 was replaced by methionine (H5- $\Delta$ F508/R553M), and in the other plasmid, R553 was replaced by glutamine (H5- $\Delta$ F508/R553Q). It is possible that other mutations within the R553-L558 region of the H5-F508 plasmid could also result in increased mating efficiency; however, the R553Q and R553M mutants were analyzed in greater detail without further mutagenesis of the R553-L558 region.

The relative mating efficiency of the  $\Delta$ F508 revertant strains are shown in Table 2, with results expressed relative to the original H5 strain. Whereas the mating efficiency of the H5- $\Delta$ F508 yeast strain is approximately 1% of the H5 strain, yeast containing the H5- $\Delta$ F508/R553Q and H5- $\Delta$ F508/R553M plasmids mated at 3% and 32%, respectively. The revertant mutations, therefore, partially suppress the  $\Delta$ F508 mating defect. The R553M mutation alone had little effect on H5 (H5-R553M); when this mutant was transformed into yeast, no further increase in mating efficiency was observed as compared with yeast containing H5 (Table 2). In accordance with this invention, mutations were found which suppress the effects of the  $\Delta$ F508 mutation.

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TABLE 2Relative Mating Efficiency of  $\Delta F508$  Revertants

<u>Genotype</u>	<u>Mating Efficiency Relative to H5 (%)</u>
H5-R553M	77.4 = 3.7
H5- $\Delta F508$ /R553M	34.2 = 7.8
H5- $\Delta F508$ /R553Q	3.2 = 0.8
H5- $\Delta F508$	1.1 = 0.5

Mating efficiencies were determined by quantitative mating assays and are expressed as a percentage relative to H5 (100%). Data are means = SEM.

EXAMPLE 2

TESTING OF THE R553M and R553Q  
MUTATIONS IN THE MAMMALIAN SYSTEM

To determine whether the mutations which corrected the  $\Delta F508$  mutation would also correct the defect in human CFTR  $\Delta F508$ , the R553Q and R553M mutations were introduced into CFTR  $\Delta F508$  cDNA and mammalian cells were transfected with these constructs to determine whether the revertant mutations would correct the defect in cAMP-regulated  $Cl^-$  transport of CFTR  $\Delta F508$ .

The revertants R553Q and R553M were tested for their effects on mislocalization and the channel gating of CFTR  $\Delta F508$ . The identified revertants modified both of these effects. Mutation of R553 to methionine partially corrected the processing of CFTR  $\Delta F508$  as assessed by three criteria; it produced fully glycosylated protein as indicated by electrophoretic separation on SDS polyacrylamide gel; it increased the appearance of the mutant protein in the plasma membrane as determined immunocytochemically; and it produced functional

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channels in the plasma membrane when measured by the SPQ halide efflux assay. These results demonstrate the functional utility of the yeast model system for identifying revertants of CF-associated mutations of the NBD1 of CFTR and its use in drug screening for pharmacologic compounds that correct the mating defect. The mutations discovered could be used to identify peptides which may be of utility in reversing the effects of the  $\Delta F508$  mutation.

### EXAMPLE 3

#### R553Q AND R553M SUPPRESS THE CFTR $\Delta F508$ ANION TRANSPORT DEFECT

The effect of the R553Q and R553M mutations on CFTR function was determined by a cAMP-stimulated halide efflux using the halide-sensitive fluorophore 6-methoxy-N-(3-sulfopropyl)-quinolinium (SPQ) (Illsley and Verkman, 1987). Expression of CFTR cDNA containing either the R553Q or the R553M mutation alone (without the  $\Delta F508$  mutation) in HeLa cells generated cAMP-stimulated halide efflux like wild-type CFTR. Cells expressing the  $\Delta F508$  allele in this recombinant system showed little, if any, cAMP-stimulated halide efflux. However, when the mutations R553Q and R553M were introduced into CFTR  $\Delta F508$  (CFTR  $\Delta F508$ /R553Q and CFTR  $\Delta F508$ /R553M, respectively), cAMP-dependent anion permeability was restored. These results indicate that the CFTR Cl<sup>-</sup> channel defect observed with the  $\Delta F508$  mutant could be suppressed by either R553 mutation.

Cl<sup>-</sup> transport by CFTR  $\Delta F508$  containing the R553Q and R553M mutations would be detected only if the processing defect of CFTR  $\Delta F508$  was suppressed. To determine further whether the suppressor

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mutations correct the processing defect associated with the  $\Delta F508$  mutation, the glycosylation patterns of CFTR and the various mutants expressed in HeLa cells were examined. CFTR is a glycoprotein that undergoes progressive glycosylation, resulting in three bands that migrate at different rates on an SDS-polyacrylamide gel. Band A is the most rapidly migrating and represents the nascent, unglycosylated protein; band B has an intermediate rate of migration and a pattern of core glycosylation consistent with processing in the endoplasmic reticulum; band C migrates most slowly and has a pattern of mature glycosylation consistent with processing in the Golgi complex. CFTR  $\Delta F508$  was only presented as the unglycosylated band A and the core glycosylated band B protein, consistent with its failure to traverse the Golgi complex and reach the plasma membrane.

We first examined the glycosylation state of CFTR in HeLa cells expressing CFTR cDNA containing either the R553Q and R553M mutations alone (without the  $\Delta F508$  mutation). Band C was present in cells expressing wild-type CFTR and also mutant CFTR containing either the R553Q or R553M mutation. In contrast, only bands A and B were present in cells expressing CFTR  $\Delta F508$ . Thus, the R553Q and R553M mutations alone do not affect the glycosylation of CFTR.

In cells transfected with the CFTR  $\Delta F508/R553M$ , a small increase in band C was detectable as compared with CFTR  $\Delta F508$ . This result was observed in three separate experiments. Band C was not able to be consistently detected in cells expressing CFTR  $\Delta F508/R553Q$  possibly owing to

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limitations in the sensitivity of the assay.

However, a detectable increase in Band C with CFTR  $\Delta F508/R553M$  as compared with CFTR  $\Delta F508$  occurred.

To assess further the ability of suppressor mutations to correct the mislocalization defect of CFTR  $\Delta F508$ , immunocytochemistry was used to detect CFTR at the cell surface in HeLa cells expressing wild-type or mutant CFTR. An antibody specific to an external epitope in the first extracellular loop of CFTR was used. (Denning, et al, 1992b). Wild-type CFTR is generally detected at the surface of unpermeabilized HeLa cells. In contrast, no CFTR was detectable at the surface of cells expressing CFTR  $\Delta F508$ . However, when cells expressed CFTR  $\Delta F508/R553M$ , CFTR was detected at the plasma membrane. For CFTR  $\Delta F508/R553Q$ , plasma membrane staining of CFTR was weak and variable and could not be demonstrated consistently. Only nonspecific staining was observed with preimmune serum or in the absence of CFTR. These results were consistent with the observation that more CFTR  $\Delta F508/R553M$  than CFTR  $\Delta F508/R553Q$  was found in the band C form and suggest that the amount of CFTR  $\Delta F508/R553Q$  at the plasma membrane is exceedingly low.

#### SINGLE-CHANNEL ANALYSIS OF CFTR $\Delta F508/R553Q$ CFTR

To determine whether the suppressor mutations altered the single-channel properties of CFTR  $\Delta F508$ , the revertant mutant CFTR  $\Delta F508/R553Q$  was expressed in HeLa cells and analyzed with the patch-clamp technique. Single-channel analysis of CFTR  $\Delta F508$  indicated that the  $P_o$  of the CFTR  $\Delta F508$   $Cl^-$  channel was reduced as compared with wild-type CFTR. Denning, et al, (1992a) reported that CFTR  $\Delta F508$  had

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a  $P_o$  of  $0.13 \pm 0.01$  (after incubation at reduced temperature), compared with a value of  $0.34 \pm 0.02$  for wild-type, and Dalemans, et al, (1991) reported that the  $P_o$  of CFTR  $\Delta F508$   $Cl^-$  channels ranged from 0.05 to 0.10, compared with a range of 0.22 to 0.35 for wild type.

As expected from the response observed in the SPQ studies, CFTR/R553Q and CFTR  $\Delta F508$ /R553Q formed functional  $Cl^-$  channels. Inside-out membrane patches from either mutant showed no  $Cl^-$  channel activity under basal conditions. However, addition of protein kinase A (PKA) and ATP to the cytosolic surface of the membrane patch activated channels in both cases; both agents were required to activate the channels ( $n = 6$  for each mutant). Single-channel events were recorded from +60 mV to -120 mV in increments of 20 mV. The channels were  $Cl^-$  selective as indicated by the reversal potentials ( $E = 20$  mV;  $E_{Cl^-} = 27$  mV). CFTR/R553Q had a single-channel slope conductance (from -120 mV to 0 mV) of  $9.8 \pm 0.5$  pS ( $n = 7$ ), and CFTR  $\Delta F508$ /R553Q had a conductance of  $10.2 \pm 0.2$  pS ( $n = 11$ ). The current measured at -100 mV for CFTR/R553Q ( $-1.1 \pm 0.1$  pA,  $n = 6$ ) and CFTR  $\Delta F508$ /R553Q ( $-1.2 \pm 0.0$  pA,  $n = 5$ ) was not different from the current measured for wild-type CFTR ( $-1.1 \pm 0.0$  pA,  $n = 7$ ). Thus, these data indicate that neither mutation altered the single-channel conductive properties of the CFTR  $Cl^-$  channel, nor did the mutations abolish the regulation by PKA. The results also indicate that, as predicted by the functional analysis of CFTR  $\Delta F508$ /R553Q by the SPQ halide efflux assay above, CFTR  $\Delta F508$ /R553Q is localized in the plasma membrane.



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Following activation with PKA and ATP, we measured the  $P_o$  for CFTR/R553Q and CFTR  $\Delta F508$ /R553Q at different MgATP concentrations. As the concentration of MgATP increased, both CFTR/R553Q and CFTR  $\Delta F508$ /R553Q spent more time in the open state. The  $P_o$  of CFTR/R553Q  $Cl^-$  channels was similar to that previously reported for wild-type CFTR  $Cl^-$  channels (Anderson and Welsh, 1992). However, at 1mM ATP, the  $P_o$  of CFTR  $\Delta F508$ /R553Q ( $0.29 \pm 0.02$ ,  $n = 7$ ) was greater than that of CFTR  $\Delta F508$   $Cl^-$  channels ( $0.13 \pm 0.01$ ,  $n = 4$ ). Thus, the R553Q mutation corrected the functional defect in gating of the CFTR  $\Delta F508$   $Cl^-$  channels.

#### YEAST MATING ASSAY WITH PLANT EXTRACT SCREENING

A suspension of yeast cells (strains A and B) was spread upon the surface of agar media in a petri dish.

The suspension consisted of equal proportions of two haploid yeast strains. Strain A - MATa, STE6::HIS3, ura3-52, lys2, trp1-298, his3-200, leu2-3,112 <plasmid H5- $\Delta F508$ , URA3, CEN>; strain B - MAT $\alpha$ , ura3-52, trp1, leu2-3,112.

Yeast strains A and B are of the a and  $\alpha$  mating types respectively, and can mate to form yeast diploids. Strain A contains the STE6/CFTR hybrid a-factor transporter gene H5- $\Delta F508$  (Teem, et al, 1993). The hybrid a-factor transporter encoded by the H5- $\Delta F508$  gene is defective as a result of the  $\Delta F508$  mutation. Strain A is therefore unable to transport the mating pheromone a-factor efficiently. Since the transport of the a-factor pheromone is required for yeast cell mating, mating between strains A and B is inefficient.

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The agar media is one rich in nutrients (nonselective for the growth of strains A and B) necessary for the optimal mating of yeast.

An extract prepared from the plant genus *Grindelia* was prepared by grinding fresh *Grindelia* leaves in a mixture of ethanol and water (1:1). This extract contains the fraction of plant material soluble in a solution of 1:1 ethanol and water. Small filter paper discs were prepared using a standard paper punch and Whatman filter paper #2. A filter paper disc containing the *Grindelia* extract was prepared by adding 10  $\mu$ l. of extract to the disc, and then allowing the disc to air dry. Additional extract was added in 10  $\mu$ l aliquots, allowing the disc to dry after each application until a total of 30  $\mu$ l. was added to the disc. The disc was then placed upon the surface of the media, on top of the yeast cells.

The mating plate was incubated for 24 hours at 30°C to allow yeast cell mating to occur.

Chemical compounds that diffuse out of the filter paper disc into the agar will come in contact with the mating cells. A component of the *Grindelia* extract promotes mating of strains A and B.

The mating plate was replica printed to a selective media plate that is incubated at 30°C for three days to allow the growth of diploid colonies.

The selective media is lacking lysine and uracil. Consequently, strains A and B cannot grow on this media (strain A has the *lys2* mutation and strain B has the *ura3-52* mutation: these mutations prevent them from growing on media without lysine and uracil respectively). However, diploids produced as a result of the fusion between strain A

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and B can grow (diploids contain the genomes of both haploids and thus contain the complementing URA3 and LYS2 wild-type alleles). Production of yeast diploid cells in the area surrounding a leaf disc would be a strong indication that the leaf disc contained a compound which corrects the  $\Delta F508$  defect. Diploids cells were easily detected by replica printing the yeast cells from the mating plate, onto another petri dish plate containing media which is selective for the growth of the diploid cells. This media will allow the diploid yeast cells to grow and form colonies, while preventing the growth of the haploid (unmated) strain A and B yeast cells. The frequency of diploid colonies produced on the selective plate is an indirect measurement of the ability of strain A to transport a-factor and mate.

A high density of yeast colonies on the selective media plate occurred in the location corresponding to the position where the filter paper disc containing the Grindelia extract occurred on the mating plate. This is clearly distinguishable from the low level of diploid colonies which are produced by the mating of the two yeast strains in the absence of a chemical compound that corrects the  $\Delta F508$  defect. (See Figure 3.)

As can be seen in Figure 3 the extract reverses the effect of the  $\Delta F508$  mutation indicating its potential for further study.

Thus it can be seen the invention accomplishes all of its objectives.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: University of Iowa Research Foundation.
- (ii) TITLE OF INVENTION: Method of Assaying for Drugs which Restore Function of CFTR Mutations
- (iii) NUMBER OF SEQUENCES: 6
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  - (F) ZIP: 50309
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
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  - (B) FILING DATE: 20-APR-1994
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  - (A) APPLICATION NUMBER: US 08/052,207
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## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3840 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..3840

(ix) FEATURE:  
 (A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1317..1488  
 (D) OTHER INFORMATION: /note= "Substituted analogous sequence from human CFTR gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA ACA GGC CTA GTG CCG GCA ATT ACT TCT ATC CTG ACG	144
Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr	
35 40 45	
GGC AGA GTG TTC GAT CTA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT	192
Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His	
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CAA GGT TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA	240
Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala	
65 70 75 80	
CTT GGT GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG	288
Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp	
85 90 95	
ATG CAC ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG	336
Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu	
100 105 110	

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GCA ATA TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA Ala Ile Cys Ala Leu Glu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu 165 170 175	528
ACT TTA ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val 180 185 190	576
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TCG ACT TTA AAT AAT ACA TTA CAC CAA ATA GTT GTT CTT CAA AAA GGC Ser Thr Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly 305 310 315 320	960

- 29 -

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Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr 1056  
340 350

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355 365

AGA CCT TCG GAA GCA GTT TTA AAG AAC GTT AGT TTA AAT TTC TCT GCA  
Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala 1152  
370 380

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Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr 1200  
385 395

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405 415

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Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Ile 1296  
420 430

GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC  
Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr 1344  
435 445

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Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr 1392  
450 460

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Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe 1440  
465 475

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Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Ile Thr Leu Ser 1488  
485 495

GGC GGG CAA CAA CAA AGA GTT GCT ATA GCA CGT GCA TTC ATC AGA GAT  
Gly Gly Gln Gln Gln Arg Val Ala Ile Ala Arg Ala Phe Ile Arg Asp 1536  
500 510

ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT  
Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val 1584  
515 525

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- 30 -

CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG 1632  
 His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys  
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 Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp  
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TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG 1728  
 Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln  
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TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC 1776  
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 580 585 590

CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT 1824  
 Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr  
 595 600 605

GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA 1872  
 Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu  
 610 615 620

ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA 1920  
 Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr  
 625 630 635 640

GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT 1968  
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AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA 2016  
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 705 710 715 720

CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA 2208  
 Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro  
 725 730 735



- 31 -

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ATT ATA TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr 865 870 875 880	2640
GAT TAT AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile 885 890 895	2688
GTC ACT AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe 900 905 910	2736
CAA TTG ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser 915 920 925	2784
AAA CGC GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile 930 935 940	2832

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- 32 -

GTC ATG TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT 2880  
 Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val 960  
 945 950

ATG ATT CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG 2928  
 Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu 975  
 965 970

TTA TTA TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC 2976  
 Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro 990  
 980 985

GAT ATA AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT 3024  
 Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu 1005  
 995 1000

GAT GAA AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA 3072  
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 1010 1015

GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC 3120  
 Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1040  
 1025 1030 1035

GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC 3168  
 Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1055  
 1045 1050

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 Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1070  
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GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA 3264  
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 Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1120  
 1105 1110 1115

GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC 3408  
 Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1135  
 1125 1130

CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT 3456  
 Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1150  
 1140 1145

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GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648
ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val 1220 1225 1230	3696
ATA ACG CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val 1235 1240 1245	3744
CTT AAA GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr 1250 1255 1260	3792
AAT AAT CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser 1265 1270 1275	3840

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1279 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg
1				5					10					15	
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile
			20					25						30	
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr
			35				40						45		

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Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His  
 50 55 60  
 Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala  
 65 70 75 80  
 Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp  
 85 90 95  
 Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu  
 100 105 110  
 Glu Ala Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu  
 115 120 125  
 Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu  
 130 135 140  
 Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val  
 145 150 155 160  
 Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu  
 165 170 175  
 Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val  
 180 185 190  
 Val Phe Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu  
 195 200 205  
 Thr Ser Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln  
 210 215 220  
 Leu Val Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys  
 225 230 235 240  
 Glu Ile Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe  
 245 250 255  
 Val Ala Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe  
 260 265 270  
 Val Gln Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu  
 275 280 285  
 Asn Ile Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly  
 290 295 300  
 Ser Thr Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly  
 305 310 315 320

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Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys  
 325 330 335  
 Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr  
 340 345 350  
 Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser  
 355 360 365  
 Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala  
 370 375 380  
 Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr  
 385 390 395 400  
 Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile  
 405 410 415  
 Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile  
 420 425 430  
 Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr  
 435 440 445  
 Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr  
 450 455 460  
 Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe  
 465 470 475 480  
 Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser  
 485 490 495  
 Gly Gly Gln Gln Gln Arg Val Ala Ile Ala Arg Ala Phe Ile Arg Asp  
 500 505 510  
 Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val  
 515 520 525  
 His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys  
 530 535 540  
 Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp  
 545 550 555 560  
 Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln  
 565 570 575  
 Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His  
 580 585 590

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Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr  
                   595                  600                  605  
 Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu  
           610                  615                  620  
 Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr  
   625                  630                  635                  640  
 Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val  
                   645                  650                  655  
 Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu  
                   660                  665                  670  
 Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser  
           675                  680  
 Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys  
   690                  695                  700  
 Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn  
   705                  710                  715                  720  
 Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro  
                   725                  730                  735  
 Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser  
                   740                  745                  750  
 Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala  
           755                  760                  765  
 Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu  
   770                  775                  780  
 Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe  
   785                  790                  795                  800  
 Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn  
                   805                  810                  815  
 Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met  
           820                  825                  830  
 Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val  
           835                  840                  845  
 Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile  
   850                  855                  860

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Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr  
 865 870 875 880  
 Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile  
 885 890 895  
 Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe  
 900 905 910  
 Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser  
 915 920 925  
 Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile  
 930 935 940  
 Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val  
 945 950 955 960  
 Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu  
 965 970 975  
 Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro  
 980 985 990  
 Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu  
 995 1000 1005  
 Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Ala Arg Thr  
 1010 1015 1020  
 Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile  
 1025 1030 1035 1040  
 Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala  
 1045 1050 1055  
 Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu  
 1060 1065 1070  
 Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu  
 1075 1080 1085  
 Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly  
 1090 1095 1100  
 Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser  
 1105 1110 1115 1120  
 Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn  
 1125 1130 1135

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Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp  
 1140 1145 1150  
 Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln  
 1155 1160 1165  
 Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala  
 1170 1175 1180  
 Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu  
 1185 1190 1195 1200  
 Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ile  
 1205 1210 1215  
 Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val  
 1220 1225 1230  
 Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val  
 1235 1240 1245  
 Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr  
 1250 1255 1260  
 Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser  
 1265 1270 1275

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3840 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..3840
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 1318..1521



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(D) OTHER INFORMATION: /note= "Substituted analogous  
sequence from human CFTR gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG	48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg	
1 5 10 15	
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA	96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile	
20 25 30	
GGT ACC GTG GCA ACA GGC CTA GTG CCG GCA ATT ACT TCT ATC CTG ACG	144
Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr	
35 40 45	
GGC AGA GTG TTC GAT CTA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT	192
Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His	
50 55 60	
CAA GGT TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA	240
Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala	
65 70 75 80	
CTT GGT GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG	288
Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp	
85 90 95	
ATG CAC ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG	336
Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu	
100 105 110	
GAG GCA TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA	384
Glu Ala Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu	
115 120 125	
AAA TTG TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA	432
Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu	
130 135 140	
AGA TCA AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT	480
Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val	
145 150 155 160	
GCA ATA TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA	528
Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu	
165 170 175	

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ACT TTA ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG 576  
Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Ala Val 190  
185  
GTG TTT TCC AGA ATG ATT CAT GTA TAT TCA GAG AAG GAG AAT TCT GAA 624  
Val Phe Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu 205  
195  
ACG AGT AAA GCA GCC CAA TTA CTT ACA TGG TCG ATG AAT GCC GCT CAA 672  
Thr Ser Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln 220  
215  
TTA GTG AGA TTA TAT TGT ACA CAA CGT CTA GAA AGG AAA AAA TTC AAG 720  
Leu Val Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys 240  
225  
GAA ATC ATA CTA AAT TGT AAC ACT TTC TTC ATC AAG AGT TGC TTT TTT 768  
Glu Ile Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe 255  
245  
GTT GCT GCA AAC GCT GGG ATC TTG AGA TTT TTG ACG TTG ACT ATG TTT 816  
Val Ala Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe 270  
260  
GTA CAG GGA TTC TGG TTT GGT TCC GCA ATG ATC AAA AAG GGC AAG CTG 864  
Val Gln Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu 285  
275  
AAC ATT AAC GAT GTA ATC ACT TGC TTC CAT TCA TGC ATT ATG CTG GGC 912  
Asn Ile Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly 300  
290  
TCG ACT TTA AAT AAT ACA TTA CAC CAA ATA GTT GTT CTT CAA AAA GGC 960  
Ser Thr Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly 320  
305  
GGA GTG GCT ATG GAA AAA ATC ATG ACT CTA TTA AAA GAT GGA TCC AAG 1008  
Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys 335  
325  
CGA AAT CCT TTA AAT AAA ACT GTA GCC CAC CAA TTT CCA CTA GAT TAT 1056  
Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr 350  
340  
GCC ACC AGT GAT CTA ACA TTT GCT AAT GTT TCG TTT TCT TAT CCA AGC 1104  
Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser 365  
355  
AGA CCT TCG GAA GCA GTT TTA AAG AAC GTT AGT TTA AAT TTC TCT GCA 1152  
Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala 380  
370

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GGA CAA TTT ACT TTC ATA GTA GGA AAA TCA GGC TCA GGT AAA TCT ACA Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr 385 390 395 400	1200
TTA TCC AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile 405 410 415	1248
TCT ATC AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile 420 425 430	1296
GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr 435 440 445	1344
ATT AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr 450 455 460	1392
AGA AGC GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe 465 470 475 480	1440
GCA GAG AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser 485 490 495	1488
GGA GGT CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA TTC ATC AGA GAT Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp 500 505 510	1536
ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val 515 520 525	1584
CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys 530 535 540	1632
ACT ACA ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp 545 550 555 560	1680
TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln 565 570 575	1728
TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His 580 585 590	1776

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CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr 595 600 605	1824
GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu 610 615 620	1872
ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr 625 630 635 640	1920
GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val 645 650 655	1968
AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu 660 665 670	2016
AAA CAA CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser 675 680 685	2064
ATT ATT CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys 690 695 700	2112
ATT CTA ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn 705 710 715 720	2160
CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro 725 730 735	2208
TCC ACG GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser 740 745 750	2256
CTT CTT GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT Leu Leu Val Leu Gly Val Ala Ala Asp Gly Ile Phe Asn Phe Ala 755 760 765	2304
AAA GGA TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu 770 775 780	2352
AGA AAT GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe 785 790 795 800	2400

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TCT GGT GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn 805 810 815	2448
GAT TTG CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met 820 825 830	2496
ACT AGT TTC GTT ACC GTA TCA ACG ATT GGA CTA ATT TGG GCG TTA GTA Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val 835 840 845	2544
TCG GGC TGG AAG TTA AGT TTG GTT TGT ATT TCG ATG TTT CCA CTC ATA Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile 850 855 860	2592
ATT ATA TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr 865 870 875 880	2640
GAT TAT AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile 885 890 895	2688
GTC ACT AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe 900 905 910	2736
CAA TTG ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser 915 920 925	2784
AAA CGC GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile 930 935 940	2832
GTC ATG TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val 945 950 955 960	2880
ATG ATT CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu 965 970 975	2928
TTA TTA TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro 980 985 990	2976
GAT ATA AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu 995 1000 1005	3024

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GAT GAA AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr 1010 1015 1020	3072
GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1025 1030 1035 1040	3120
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1045 1050 1055	3168
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1060 1065 1070	3216
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu 1075 1080 1085	3264
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly 1090 1095 1100	3312
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1105 1110 1115 1120	3360
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1125 1130 1135	3408
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1140 1145 1150	3456
GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648

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ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT 3696  
 Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val  
 1220 1225 1230  
 ATA ACG CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT 3744  
 Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val  
 1235 1240 1245  
 CTT AAA GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT 3792  
 Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr  
 1250 1255 1260  
 AAT AAT CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA 3840  
 Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser  
 1265 1270 1275

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1279 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg  
 1 5 10 15  
 Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile  
 20 25 30  
 Gly Thr Val Ala Thr Gly Leu Val Pro Ala Ile Thr Ser Ile Leu Thr  
 35 40 45  
 Gly Arg Val Phe Asp Leu Leu Ser Val Phe Val Ala Asn Gly Ser His  
 50 55 60  
 Gln Gly Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala  
 65 70 75 80  
 Leu Gly Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp  
 85 90 95  
 Met His Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu  
 100 105 110  
 Glu Ala Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu  
 115 120 125

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Lys Leu Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu  
 130 135 140  
 Arg Ser Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val  
 145 150 155 160  
 Ala Ile Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu  
 165 170 175  
 Thr Leu Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val  
 180 185 190  
 Val Phe Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu  
 195 200 205  
 Thr Ser Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln  
 210 215 220  
 Leu Val Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys  
 225 230 235 240  
 Glu Ile Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe  
 245 250 255  
 Val Ala Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe  
 260 265 270  
 Val Gln Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu  
 275 280 285  
 Asn Ile Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly  
 290 295 300  
 Ser Thr Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly  
 305 310 315 320  
 Gly Val Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys  
 325 330 335  
 Arg Asn Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr  
 340 345 350  
 Ala Thr Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser  
 355 360 365  
 Arg Pro Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala  
 370 375 380  
 Gly Gln Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr  
 385 390 395 400



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Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile  
 405 410 415  
 Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile  
 420 425 430  
 Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr  
 435 440 445  
 Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr  
 450 455 460  
 Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe  
 465 470 475 480  
 Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser  
 485 490 495  
 Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp  
 500 505 510  
 Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val  
 515 520 525  
 His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys  
 530 535 540  
 Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp  
 545 550 555 560  
 Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln  
 565 570 575  
 Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His  
 580 585 590  
 Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr  
 595 600 605  
 Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu  
 610 615 620  
 Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr  
 625 630 635 640  
 Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val  
 645 650 655  
 Arg Thr Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu  
 660 665 670

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Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser  
 675 680 685  
 Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys  
 690 695 700  
 Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn  
 705 710 715 720  
 Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro  
 725 730 735  
 Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser  
 740 745 750  
 Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala  
 755 760 765  
 Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu  
 770 775 780  
 Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe  
 785 790 795 800  
 Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn  
 805 810 815  
 Asp Leu Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met  
 820 825 830  
 Thr Ser Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val  
 835 840 845  
 Ser Gly Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile  
 850 855 860  
 Ile Ile Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr  
 865 870 875 880  
 Asp Tyr Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile  
 885 890 895  
 Val Thr Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe  
 900 905 910  
 Gln Leu Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser  
 915 920 925  
 Lys Arg Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile  
 930 935 940

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Val Met Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val  
 945 950 955 960  
 Met Ile His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu  
 965 970 975  
 Leu Leu Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro  
 980 985 990  
 Asp Ile Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu  
 995 1000 1005  
 Asp Glu Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr  
 1010 1015 1020  
 Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile  
 1025 1030 1035 1040  
 Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala  
 1045 1050 1055  
 Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu  
 1060 1065 1070  
 Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu  
 1075 1080 1085  
 Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly  
 1090 1095 1100  
 Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser  
 1105 1110 1115 1120  
 Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn  
 1125 1130 1135  
 Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp  
 1140 1145 1150  
 Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln  
 1155 1160 1165  
 Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala  
 1170 1175 1180  
 Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu  
 1185 1190 1195 1200  
 Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ile  
 1205 1210 1215

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Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val
      1220                      1225                      1230
Ile Thr His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val
      1235                      1240                      1245
Leu Lys Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr
      1250                      1255                      1260
Asn Asn Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser
      1265                      1270                      1275

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## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 3786 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic).

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..3786

(ix) FEATURE:  
 (A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1264..1524  
 (D) OTHER INFORMATION: /note= "Substituted analogous  
 sequence from human CFTR gene"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATG AAC TTT TTA AGT TTT AAG ACT ACA AAA CAC TAT CAC ATT TTC AGG      48
Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg
  1              5              10              15
TAC GTG AAC ATA CGG AAT GAC TAC AGG CTG TTA ATG ATA ATG ATA ATA      96
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile
      20              25              30

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GGT ACC GTG GCA CTA TCA GTT TTC GTG GCT AAT GGG TCA CAT CAA GGT	144
Gly Thr Val Ala Leu Ser Val Phe Val Ala Asn Gly Ser His Gln Gly	
35 40 45	
TTG TAT TCC CAA CTA GTA CAG AGG TCA ATG GCA GTA ATG GCA CTT GGT	192
Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala Leu Gly	
50 55 60	
GCG GCT TCT GTG CCA GTA ATG TGG CTT TCT CTA ACA AGT TGG ATG CAC	240
Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp Met His	
65 70 75 80	
ATC GGC GAG AGA CAA GGC TTT AGA ATA CGG TCA CAG ATA TTG GAG GCA	288
Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu Glu Ala	
85 90 95	
TAT TTG GAG GAA AAG CCA ATG GAA TGG TAC GAC AAT AAT GAA AAA TTG	336
Tyr Leu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu Lys Leu	
100 105 110	
TTA GGA GAT TTT ACT CAA ATC AAC AGA TGT GTG GAA GAG CTA AGA TCA	384
Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu Arg Ser	
115 120 125	
AGC TCC GCA GAG GCA TCA GCC ATA ACT TTC CAG AAT TTA GTT GCA ATA	432
Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val Ala Ile	
130 135 140	
TGT GCG CTT CTG GGG ACG TCA TTC TAC TAT TCT TGG TCA TTA ACT TTA	480
Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu Thr Leu	
145 150 155 160	
ATT ATT CTT TGC AGC TCT CCA ATA ATC ACA TTT TTT GCA GTG GTG TTT	528
Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val Val Phe	
165 170 175	
TCC AGA ATG ATT CAT GTA TAT TCA GAG AAG GAG AAT TCT GAA ACG AGT	576
Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu Thr Ser	
180 185 190	
AAA GCA GCC CAA TTA CTT ACA TGG TCG ATG AAT GCC GCT CAA TTA GTG	624
Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln Leu Val	
195 200 205	
AGA TTA TAT TGT ACA CAA CGT CTA GAA AGG AAA AAA TTC AAG GAA ATC	672
Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys Glu Ile	
210 215 220	
ATA CTA AAT TGT AAC ACT TTC TTC ATC AAG AGT TGC TTT TTT GTT GCT	720
Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe Val Ala	
225 230 235 240	

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GCA AAC GCT GGG ATC TTG AGA TTT TTG ACG TTG ACT ATG TTT GTA CAG Ala Asn Ala Gly Ile Leu Arg Phe Leu Thr Leu Thr Met Phe Val Gln 245 250 255	768
GGA TTC TGG TTT GGT TCC GCA ATG ATC AAA AAG GGC AAG CTG AAC ATT Gly Phe Trp Phe Gly Ser Ala Met Ile Lys Lys Gly Lys Leu Asn Ile 260 265 270	816
AAC GAT GTA ATC ACT TGC TTC CAT TCA TGC ATT ATG CTG GGC TCG ACT Asn Asp Val Ile Thr Cys Phe His Ser Cys Ile Met Leu Gly Ser Thr 275 280 285	864
TTA AAT AAT ACA TTA CAC CAA ATA GTT GTT CTT CAA AAA GGC GGA GTG Leu Asn Asn Thr Leu His Gln Ile Val Val Leu Gln Lys Gly Gly Val 290 295 300	912
GCT ATG GAA AAA ATC ATG ACT CTA TTA AAA GAT GGA TCC AAG CGA AAT Ala Met Glu Lys Ile Met Thr Leu Leu Lys Asp Gly Ser Lys Arg Asn 305 310 315 320	960
CCT TTA AAT AAA ACT GTA GCC CAC CAA TTT CCA CTA GAT TAT GCC ACC Pro Leu Asn Lys Thr Val Ala His Gln Phe Pro Leu Asp Tyr Ala Thr 325 330 335	1008
AGT GAT CTA ACA TTT GCT AAT GTT TCG TTT TCT TAT CCA AGC AGA CCT Ser Asp Leu Thr Phe Ala Asn Val Ser Phe Ser Tyr Pro Ser Arg Pro 340 345 350	1056
TCG GAA GCA GTT TTA AAG AAC GTT AGT TTA AAT TTC TCT GCA GGA CAA Ser Glu Ala Val Leu Lys Asn Val Ser Leu Asn Phe Ser Ala Gly Gln 355 360 365	1104
TTT ACT TTC ATA GTA GGA AAA TCA GGC TCA GGT AAA TCT ACA TTA TCC Phe Thr Phe Ile Val Gly Lys Ser Gly Ser Gly Lys Ser Thr Leu Ser 370 375 380	1152
AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA TCT ATC Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile Ser Ile 385 390 395 400	1200
AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT GAA AAT Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile Glu Asn 405 410 415	1248
ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC ATT AAA Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr Ile Lys 420 425 430	1296
GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC AGA AGC Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser 435 440 445	1344

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GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT GCA GAG Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu 450 455 460	1392
AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT GGA GGT Lys Asp Asn Ile Val Leu Gly Glu Gly Ile Thr Leu Ser Gly Gly 465 470 475 480	1440
CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA GTA TAC AAA GAT GCT GAT Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp 485 490 495	1488
TTG TAT TTA TTA GAC TCT CCT TTT GGA TAC CTA GAT ATT GTT CAT CGC Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr Leu Asp Ile Val His Arg 500 505 510	1536
AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG ACT ACA Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys Thr Thr 515 520 525	1584
ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC TAT TTA Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp Tyr Leu 530 535 540	1632
TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG TCT GAA Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln Ser Glu 545 550 555 560	1680
CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC CTA CAG Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His Leu Gln 565 570 575	1728
AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT GAA GAA Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr Glu Glu 580 585 590	1776
AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA ACA CCA Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu Thr Pro 595 600 605	1824
AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA GAT CAG Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr Asp Gln 610 615 620	1872
TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT AGA ACA Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val Arg Thr 625 630 635 640	1920
AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA AAA CAA Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu Lys Gln 645 650 655	1968

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CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC ATT ATT Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser Ile Ile 660 665 670	2016
CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA ATT CTA Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys Ile Leu 675 680 685	2064
ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT CCC GTC Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn Pro Val 690 695 700	2112
TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA TCC ACG Phe Ser Tyr Thr Phe Ser Phe Leu Leu Gly Ile Val Pro Ser Thr 705 710 715 720	2160
GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG CTT CTT Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser Leu Leu 725 730 735	2208
GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT AAA GGA Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala Lys Gly 740 745 750	2256
TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT AGA AAT Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu Arg Asn 755 760 765	2304
GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT TCT GGT Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe Ser Gly 770 775 780	2352
GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT GAT TTG Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn Asp Leu 785 790 800	2400
CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG ACT AGT Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met Thr Ser 805 810 815	2448
TTC GTT ACC GTA TCA ACG ATT GGA CTA ATT TGG GCG TTA GTA TCG GGC Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val Ser Gly 820 825 830	2496
TGG AAG TTA AGT TTG GTT TGT ATT TCG ATG TTT CCA CTC ATA ATT ATA Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile Ile Ile 835 840 845	2544
TTT TCA GCA ATA TAT GGA GGC ATT TTA CAA AAA TGC GAA ACA GAT TAT Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr Asp Tyr 850 855 860	2592



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AAG ACA TCT GTT GCT CAG TTA GAA AAC TGC CTG TAC CAG ATT GTC ACT Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile Val Thr 865 870 875 880	2640
AAC ATT AAA ACC ATT AAG TGC TTA CAA GCT GAA TTT CAT TTT CAA TTG Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe Gln Leu 885 890 895	2688
ACC TAC CAT GAC TTG AAG ATA AAA ATG CAA CAA ATT GCC TCC AAA CGC Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser Lys Arg 900 905 910	2736
GCC ATT GCC ACA GGA TTT GGT ATA TCT ATG ACA AAC ATG ATT GTC ATG Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile Val Met 915 920 925	2784
TGT ATC CAA GCT ATT ATT TAC TAC TAT GGC CTA AAG CTG GTT ATG ATT Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val Met Ile 930 935 940	2832
CAC GAG TAC ACC TCA AAG GAA ATG TTT ACG ACT TTC ACT TTG TTA TTA His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu Leu Leu 945 950 955 960	2880
TTC ACT ATT ATG TCA TGC ACT AGC CTA GTA AGT CAG ATA CCC GAT ATA Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro Asp Ile 965 970 975	2928
AGT AGA GGC CAA CGT GCT GCC AGT TGG ATC TAT AGG ATT CTT GAT GAA Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu Asp Glu 980 985 990	2976
AAG CAT AAT ACC CTA GAG GTT GAA AAC AAT AAT GCT AGA ACA GTG GGA Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr Val Gly 995 1000 1005	3024
ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC GTT TCA Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile Val Ser 1010 1015 1020	3072
ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC TTT GTT Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val 1025 1030 1035 1040	3120
TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA GGT ATC Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile 1045 1050 1055	3168
ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA TTA ACA Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Thr 1060 1065 1070	3216

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AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT ACG GAC Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp 1075 1080 1085	3264
GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA GTG GTT Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val 1090 1095 1100	3312
GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC CTA ACT Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr 1105 1110 1115 1120	3360
TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT GCA TTA Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu 1125 1130 1135	3408
AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG GGC TTG Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu 1140 1145 1150	3456
GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG CAA AGG Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg 1155 1160 1165	3504
CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG ATT TTA Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu 1170 1175 1180	3552
GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC ATC AAT Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile Ile Asn 1185 1190 1195 1200	3600
GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT ATA ACG Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr 1205 1210 1215	3648
CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT CTT AAA His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys 1220 1225 1230	3696
GAT GGT AAA GTG GTT GAG CGA GGT AAC TTC GAC ACT TTA TAT AAT AAT Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr Asn Asn 1235 1240 1245	3744
CGC GGG GAA TTA TTC CAA ATT GTT TCC AAC CAA AGC AGT TAA Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser 1250 1255 1260	3786

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 1261 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asn Phe Leu Ser Phe Lys Thr Thr Lys His Tyr His Ile Phe Arg
 1           5           10           15
Tyr Val Asn Ile Arg Asn Asp Tyr Arg Leu Leu Met Ile Met Ile Ile
      20           25           30
Gly Thr Val Ala Leu Ser Val Phe Val Ala Asn Gly Ser His Gln Gly
      35           40           45
Leu Tyr Ser Gln Leu Val Gln Arg Ser Met Ala Val Met Ala Leu Gly
      50           55           60
Ala Ala Ser Val Pro Val Met Trp Leu Ser Leu Thr Ser Trp Met His
      65           70           75           80
Ile Gly Glu Arg Gln Gly Phe Arg Ile Arg Ser Gln Ile Leu Glu Ala
      85           90           95
Tyr Leu Glu Glu Lys Pro Met Glu Trp Tyr Asp Asn Asn Glu Lys Leu
      100          105          110
Leu Gly Asp Phe Thr Gln Ile Asn Arg Cys Val Glu Glu Leu Arg Ser
      115          120          125
Ser Ser Ala Glu Ala Ser Ala Ile Thr Phe Gln Asn Leu Val Ala Ile
      130          135          140
Cys Ala Leu Leu Gly Thr Ser Phe Tyr Tyr Ser Trp Ser Leu Thr Leu
      145          150          155          160
Ile Ile Leu Cys Ser Ser Pro Ile Ile Thr Phe Phe Ala Val Val Phe
      165          170          175
Ser Arg Met Ile His Val Tyr Ser Glu Lys Glu Asn Ser Glu Thr Ser
      180          185          190
Lys Ala Ala Gln Leu Leu Thr Trp Ser Met Asn Ala Ala Gln Leu Val
      195          200          205
Arg Leu Tyr Cys Thr Gln Arg Leu Glu Arg Lys Lys Phe Lys Glu Ile
      210          215          220
Ile Leu Asn Cys Asn Thr Phe Phe Ile Lys Ser Cys Phe Phe Val Ala
      225          230          235          240

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Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	Val	Gln
				245						250				255	
Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	Asn	Ile
			260					265					270		
Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	Ser	Thr
		275					280					285			
Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	Gly	Val
	290					295					300				
Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	Arg	Asn
305					310					315					320
Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	Ala	Thr
				325					330					335	
Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	Arg	Pro
			340					345					350		
Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	Gly	Gln
		355					360					365			
Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	Leu	Ser
	370					375					380				
Asn	Leu	Leu	Leu	Arg	Phe	Tyr	Asp	Gly	Tyr	Asn	Gly	Ser	Ile	Ser	Ile
385					390					395					400
Asn	Gly	His	Asn	Ile	Gln	Thr	Ile	Asp	Gln	Lys	Leu	Leu	Ile	Glu	Asn
				405					410					415	
Ile	Thr	Val	Val	Glu	Gln	Phe	Ser	Trp	Ile	Met	Pro	Gly	Thr	Ile	Lys
			420					425					430		
Glu	Asn	Ile	Ile	Phe	Gly	Val	Ser	Tyr	Asp	Glu	Tyr	Arg	Tyr	Arg	Ser
		435					440					445			
Val	Ile	Lys	Ala	Cys	Gln	Leu	Glu	Glu	Asp	Ile	Ser	Lys	Phe	Ala	Glu
	450					455					460				
Lys	Asp	Asn	Ile	Val	Leu	Gly	Glu	Gly	Gly	Ile	Thr	Leu	Ser	Gly	Gly
465					470					475					480
Gln	Arg	Ala	Arg	Ile	Ser	Leu	Ala	Arg	Ala	Val	Tyr	Lys	Asp	Ala	Asp
				485					490					495	
Leu	Tyr	Leu	Leu	Asp	Ser	Pro	Phe	Gly	Tyr	Leu	Asp	Ile	Val	His	Arg
			500					505					510		

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Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys Thr Thr  
 515 520 525  
 Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp Tyr Leu  
 530 535 540  
 Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln Ser Glu  
 545 550 555 560  
 Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His Leu Gln  
 565 570 575  
 Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr Glu Glu  
 580 585 590  
 Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu Thr Pro  
 595 600 605  
 Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr Asp Gln  
 610 615 620  
 Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val Arg Thr  
 625 630 635 640  
 Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu Lys Gln  
 645 650 655  
 Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser Ile Ile  
 660 665 670  
 Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys Ile Leu  
 675 680 685  
 Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn Pro Val  
 690 695 700  
 Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro Ser Thr  
 705 710 715 720  
 Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser Leu Leu  
 725 730 735  
 Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala Lys Gly  
 740 745 750  
 Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu Arg Asn  
 755 760 765  
 Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe Ser Gly  
 770 775 780

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Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn Asp Leu  
 785 790 795 800  
 Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met Thr Ser  
 805 810 815  
 Phe Val Thr Val Ser Thr Ile Gly Leu Ile Trp Ala Leu Val Ser Gly  
 820 825 830  
 Trp Lys Leu Ser Leu Val Cys Ile Ser Met Phe Pro Leu Ile Ile Ile  
 835 840 845  
 Phe Ser Ala Ile Tyr Gly Gly Ile Leu Gln Lys Cys Glu Thr Asp Tyr  
 850 855 860  
 Lys Thr Ser Val Ala Gln Leu Glu Asn Cys Leu Tyr Gln Ile Val Thr  
 865 870 875 880  
 Asn Ile Lys Thr Ile Lys Cys Leu Gln Ala Glu Phe His Phe Gln Leu  
 885 890 895  
 Thr Tyr His Asp Leu Lys Ile Lys Met Gln Gln Ile Ala Ser Lys Arg  
 900 905 910  
 Ala Ile Ala Thr Gly Phe Gly Ile Ser Met Thr Asn Met Ile Val Met  
 915 920 925  
 Cys Ile Gln Ala Ile Ile Tyr Tyr Tyr Gly Leu Lys Leu Val Met Ile  
 930 935 940  
 His Glu Tyr Thr Ser Lys Glu Met Phe Thr Thr Phe Thr Leu Leu Leu  
 945 950 955 960  
 Phe Thr Ile Met Ser Cys Thr Ser Leu Val Ser Gln Ile Pro Asp Ile  
 965 970 975  
 Ser Arg Gly Gln Arg Ala Ala Ser Trp Ile Tyr Arg Ile Leu Asp Glu  
 980 985 990  
 Lys His Asn Thr Leu Glu Val Glu Asn Asn Asn Ala Arg Thr Val Gly  
 995 1000 1005  
 Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile Val Ser  
 1010 1015 1020  
 Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val  
 1025 1030 1035 1040  
 Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile  
 1045 1050 1055

- 61 -

Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Leu Thr  
 1060 1065 1070  
 Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp  
 1075 1080 1085  
 Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val  
 1090 1095 1100  
 Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr  
 1105 1110 1115 1120  
 Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu  
 1125 1130 1135  
 Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu  
 1140 1145 1150  
 Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg  
 1155 1160 1165  
 Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu  
 1170 1175 1180  
 Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile Ile Asn  
 1185 1190 1195 1200  
 Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr  
 1205 1210 1215  
 His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys  
 1220 1225 1230  
 Asp Gly Lys Val Val Glu Arg Gly Asn Phe Asp Thr Leu Tyr Asn Asn  
 1235 1240 1245  
 Arg Gly Glu Leu Phe Gln Ile Val Ser Asn Gln Ser Ser  
 1250 1255 1260

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What is claimed is:

1. A method of identifying potential treatments which correct mislocalization and/or misfunction of CFTR resulting from mutations said method comprising: forming a chimeric gene sequence by substituting a portion of a gene sequence which encodes the first nucleotide binding domain of cystic fibrosis conductance regulator with a gene sequence which encodes STE6; said CFTR sequence being a CFTR gene sequence encoding a plurality of amino acids from amino acid 444 to and including amino acid 577, such that introduction of a mutation in the CFTR region inhibits expression of the chimeric STE6 gene product; transforming said chimeric gene sequence into a yeast strain with no functional STE6 gene; introducing said potential treatment to said yeast strain; and assaying for the production of diploid yeast colonies following yeast cell mating.
2. The method of claim 1 wherein said mutation is a deletion of phenylalanine at position 508 of the CFTR gene product.
3. The method of claim 1 wherein said CFTR substitute is the gene sequence which encodes from amino acid 494 through 546 of the CFTR gene product (SEQ ID #1).
4. The method of claim 1 wherein said CFTR substitution is the gene sequence which encodes from amino acid 494 up to and including amino acid 558 of the CFTR gene product (SEQ ID #3).



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5. The method of claim 1 wherein said CFTR substitution is the gene sequence which encodes from amino acid 494 up to and including 577 of the CFTR gene product (SEQ ID #5).

6. The method of claim 3, 4, or 5 wherein said CFTR substitution includes a mutation which causes cystic fibrosis.

7. A chimeric protein comprising a yeast STE6 protein product having substituted therein an analogous portion of CFTR nucleotide binding domain including a plurality of amino acids from amino acid 443 to amino acid 577.

8. The protein of claim 7 wherein said CFTR sequence includes a mutation which results in cystic fibrosis.

9. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 2.

10. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 4.

11. The protein of claim 7 wherein said amino acid sequence is SEQ ID # 6.

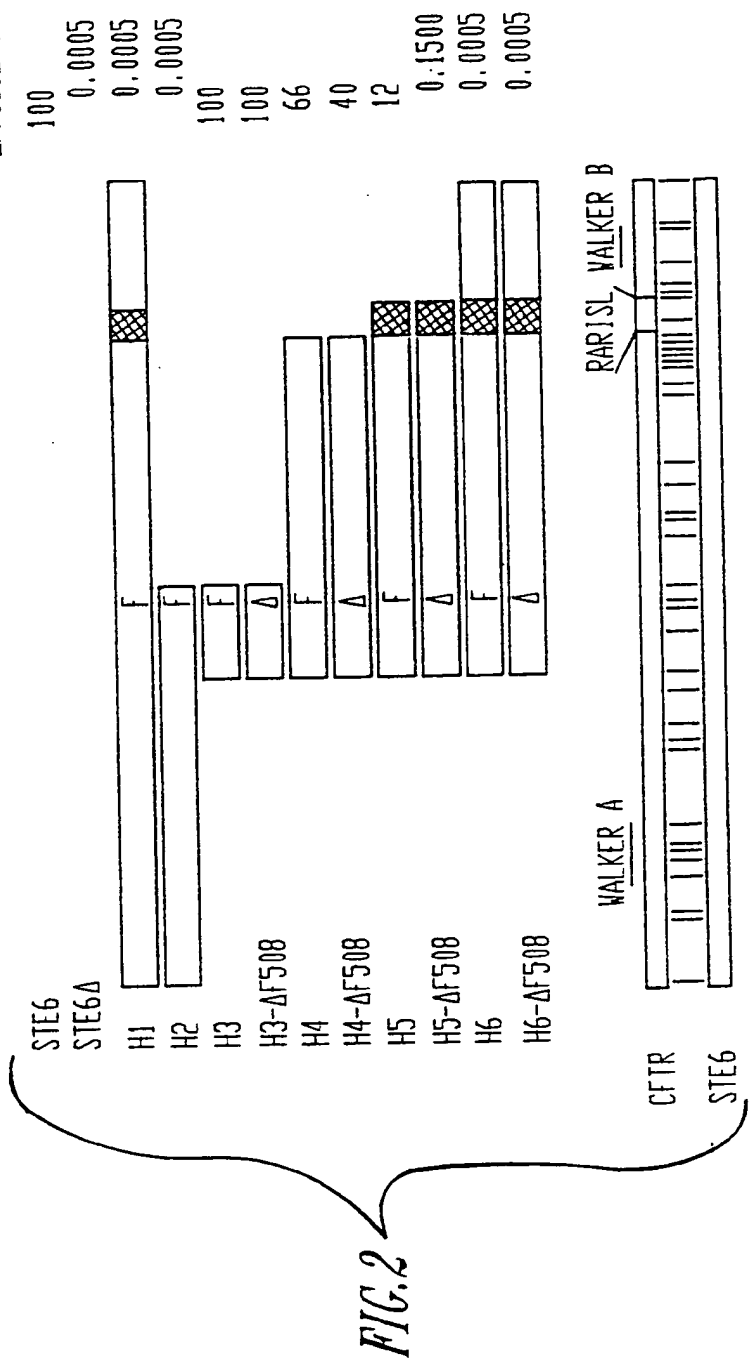
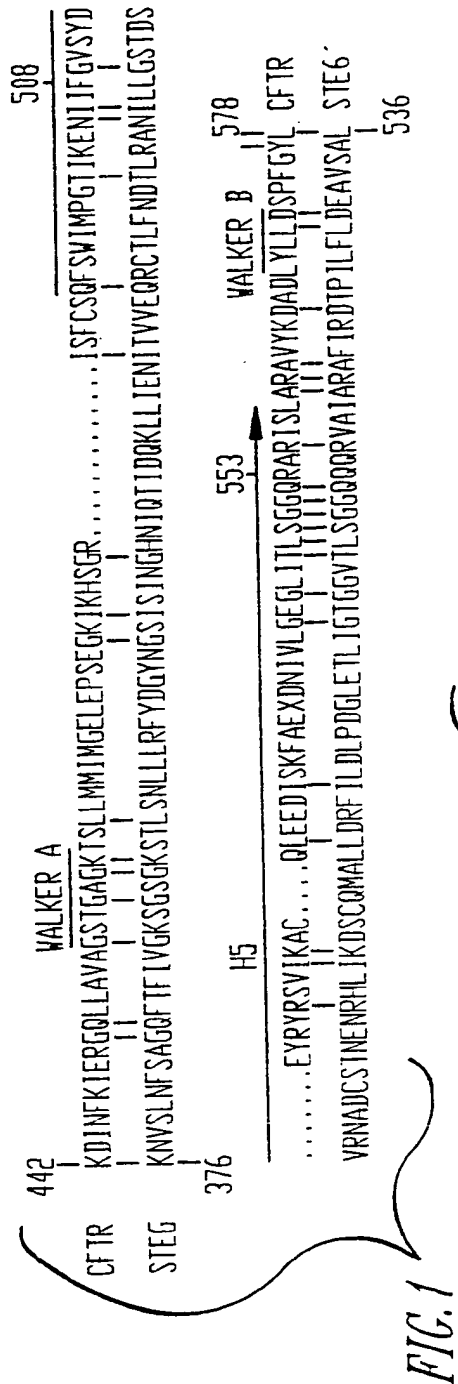
12. A chimeric protein sufficiently homologous to that of claim 7 such that introduction of a mutation in the CFTR portion will prevent transport of a-factor out a yeast cell thereby preventing yeast mating.

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13. A DNA sequence which encodes the protein of claim 7.

14. A host cell transformed with the DNA sequence of claim 13 in a manner allowing expression of the STE6 gene.

15. A method of screening for potential inhibitors of ABC transporters by the use of STE6 chimeras, said method comprising selecting from the class of related proteins consisting essentially of multi-drug resistance (mdr) or P-glycoprotein, the chloroquine resistance transporter (pfmdr) of Plasmodium falciparum, and CFTR; substituting the nucleotide sequence of the STE6 gene with the sequence encoding the analogous portion of said ABC transporter; transforming said chimeric gene sequence into a yeast which is deleted for the STE6 gene; introducing a treatment to said transformed yeast strain; and assaying for the production of yeast diploid colonies following yeast cell mating.



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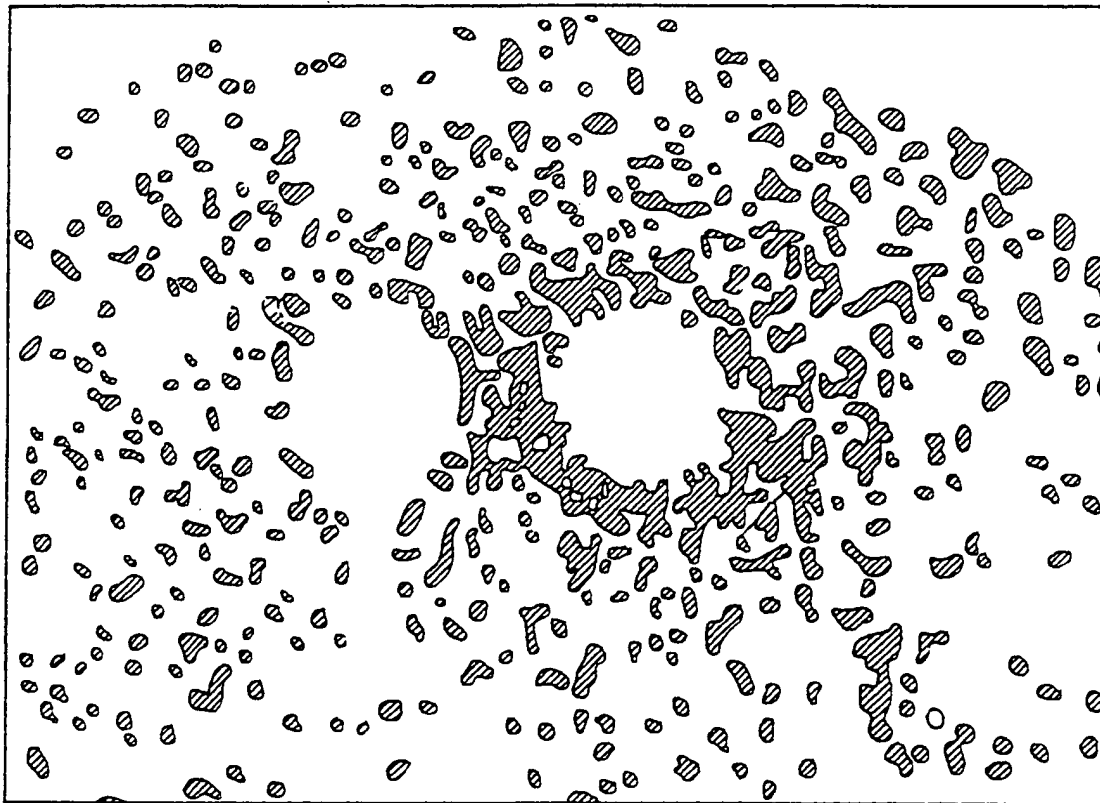
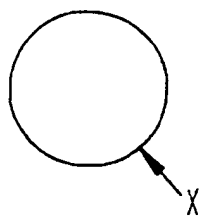
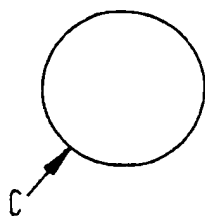


FIG. 3



X=EXTRACT FROM GRINDELIA  
C=CONTROL

SELECTIVE MEDIA PLATE WITH DIPLOID COLONIES

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## H4 Chimera (Seq. ID No. 1)

ATG	AAC	TTT	TTA	AGT	TTT	AAG	ACT	ACA	AAA	CAC	TAT	CAC	ATT	TTC	AGG	48
Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg	
1				5					10					15		
TAC	GTG	AAC	ATA	CGG	AAT	GAC	TAC	AGG	CTG	TTA	ATG	ATA	ATG	ATA	ATA	96
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile	
			20					25					30			
GGT	ACC	GTG	GCA	ACA	GGC	CTA	GTG	CCG	GCA	ATT	ACT	TCT	ATC	CTG	ACG	144
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr	
		35				40						45				
GGC	AGA	GTG	TTC	GAT	CTA	CTA	TCA	GTT	TTC	GTG	GCT	AAT	GGG	TCA	CAT	192
Gly	Arg	Val	Phe	Asp	Leu	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His	
	50					55					60					
CAA	GGT	TTG	TAT	TCC	CAA	CTA	GTA	CAG	AGG	TCA	ATG	GCA	GTA	ATG	GCA	240
Gln	Gly	Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala	
65					70				75						80	
CTT	GGT	GCG	GCT	TCT	GTG	CCA	GTA	ATG	TGG	CTT	TCT	CTA	ACA	AGT	TGG	288
Leu	Gly	Ala	Ala	Ser	Val	Pro	Val	Met	Trp	Leu	Ser	Leu	Thr	Ser	Trp	
				85					90					95		
ATG	CAC	ATC	GGC	GAG	AGA	CAA	GGC	TTT	AGA	ATA	CGG	TCA	CAG	ATA	TTG	336
Met	His	Ile	Gly	Glu	Arg	Gln	Gly	Phe	Arg	Ile	Arg	Ser	Gln	Ile	Leu	
			100					105					110			
GAG	GCA	TAT	TTG	GAG	GAA	AAG	CCA	ATG	GAA	TGG	TAC	GAC	AAT	AAT	GAA	384
Glu	Ala	Tyr	Leu	Glu	Glu	Lys	Pro	Met	Glu	Trp	Tyr	Asp	Asn	Asn	Glu	
		115					120					125				
AAA	TTG	TTA	GGA	GAT	TTT	ACT	CAA	ATC	AAC	AGA	TGT	GTG	GAA	GAG	CTA	432
Lys	Leu	Leu	Gly	Asp	Phe	Thr	Gln	Ile	Asn	Arg	Cys	Val	Glu	Glu	Leu	
	130					135					140					
AGA	TCA	AGC	TCC	GCA	GAG	GCA	TCA	GCC	ATA	ACT	TTC	CAG	AAT	TTA	GTT	480
Arg	Ser	Ser	Ser	Ala	Glu	Ala	Ser	Ala	Ile	Thr	Phe	Gln	Asn	Leu	Val	
145					150					155					160	
GCA	ATA	TGT	GCG	CTT	CTG	GGG	ACG	TCA	TTC	TAC	TAT	TCT	TGG	TCA	TTA	528
Ala	Ile	Cys	Ala	Leu	Leu	Gly	Thr	Ser	Phe	Tyr	Tyr	Ser	Trp	Ser	Leu	
			165						170				175			
ACT	TTA	ATT	ATT	CTT	TGC	AGC	TCT	CCA	ATA	ATC	ACA	TTT	TTT	GCA	GTG	576
Thr	Leu	Ile	Ile	Leu	Cys	Ser	Ser	Pro	Ile	Ile	Thr	Phe	Phe	Ala	Val	
			180					185					190			

FIG. 4A

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GTG	TTT	TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	624
Val	Phe	Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	
		195					200					205				
ACG	AGT	AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	672
Thr	Ser	Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	
	210					215				220						
TTA	GTG	AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	720
Leu	Val	Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	
225					230					235					240	
GAA	ATC	ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	768
Glu	Ile	Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	
				245					250					255		
GTT	GCT	GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	816
Val	Ala	Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	
			260					265					270			
GTA	CAG	GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	864
Val	Gln	Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	
		275					280					285				
AAC	ATT	AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	912
Asn	Ile	Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	
	290					295					300					
TCG	ACT	TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	960
Ser	Thr	Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	
305					310					315					320	
GGA	GTG	GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	1008
Gly	Val	Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	
				325					330					335		
CGA	AAT	CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	1056
Arg	Asn	Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	
			340					345					350			
GCC	ACC	AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	1104
Ala	Thr	Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	
		355					360					365				
AGA	CCT	TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	1152
Arg	Pro	Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	
	370					375					380					
GGA	CAA	TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	1200
Gly	Gln	Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	
385					390					395					400	

FIG. 4B

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TTA	TCC	AAC	TTA	TTA	TTA	AGG	TTC	TAC	GAT	GGC	TAT	AAT	GGA	TCG	ATA	1248
Leu	Ser	Asn	Leu	Leu	Leu	Arg	Phe	Tyr	Asp	Gly	Tyr	Asn	Gly	Ser	Ile	
				405					410					415		
TCT	ATC	AAT	GGC	CAC	AAT	ATC	CAA	ACA	ATC	GAC	CAA	AAA	TTG	CTA	ATT	1296
Ser	Ile	Asn	Gly	His	Asn	Ile	Gln	Thr	Ile	Asp	Gln	Lys	Leu	Leu	Ile	
			420					425					430			
GAA	AAT	ATC	ACC	GTC	GTA	GAA	CAG	TTT	TCC	TGG	ATT	ATG	CCT	GGC	ACC	1344
Glu	Asn	Ile	Thr	Val	Val	Glu	Gln	Phe	Ser	Trp	Ile	Met	Pro	Gly	Thr	
		435					440					445				
					STE6			CFTR								
ATT	AAA	GAA	AAT	ATC	ATC	TTT	GGT	GTT	TCC	TAT	GAT	GAA	TAT	AGA	TAC	1392
Ile	Lys	Glu	Asn	Ile	Ile	Phe	Gly	Val	Ser	Tyr	Asp	Glu	Tyr	Arg	Tyr	
	450					455					460					
AGA	AGC	GTC	ATC	AAA	GCA	TGC	CAA	CTA	GAA	GAG	GAC	ATC	TCC	AAG	TTT	1440
Arg	Ser	Val	Ile	Lys	Ala	Cys	Gln	Leu	Glu	Glu	Asp	Ile	Ser	Lys	Phe	
	465				470					475					480	
GCA	GAG	AAA	GAC	AAT	ATA	GTT	CTT	GGA	GAA	GGT	GGA	ATC	ACA	CTG	AGT	1488
Ala	Glu	Lys	Asp	Asn	Ile	Val	Leu	Gly	Glu	Gly	Gly	Ile	Thr	Leu	Ser	
			485					490						495		
														CFTR	STE6	
GGC	GGG	CAA	CAA	CAA	AGA	GTT	GCT	ATA	GCA	CGT	GCA	TTC	ATC	AGA	GAT	1536
Gly	Gly	Gln	Gln	Gln	Arg	Val	Ala	Ile	Ala	Arg	Ala	Phe	Ile	Arg	Asp	
			500				505						510			
ACT	CCA	ATA	TTA	TTC	TTA	GAC	GAA	GCT	GTA	TCG	GCT	CTA	GAT	ATT	GTT	1584
Thr	Pro	Ile	Leu	Phe	Leu	Asp	Glu	Ala	Val	Ser	Ala	Leu	Asp	Ile	Val	
		515					520					525				
CAT	CGC	AAC	CTG	TTG	ATG	AAG	GCA	ATT	AGG	CAT	TGG	AGG	AAA	GGA	AAG	1632
His	Arg	Asn	Leu	Leu	Met	Lys	Ala	Ile	Arg	His	Trp	Arg	Lys	Gly	Lys	
	530					535					540					
ACT	ACA	ATC	ATA	TTG	ACG	CAT	GAG	TTG	AGC	CAA	ATT	GAA	TCT	GAT	GAC	1680
Thr	Thr	Ile	Ile	Leu	Thr	His	Glu	Leu	Ser	Gln	Ile	Glu	Ser	Asp	Asp	
	545				550					555					560	
TAT	TTA	TAT	TTA	ATG	AAG	GAA	GGT	GAA	GTT	GTT	GAA	AGC	GGC	ACC	CAG	1728
Tyr	Leu	Tyr	Leu	Met	Lys	Glu	Gly	Glu	Val	Val	Glu	Ser	Gly	Thr	Gln	
			565					570						575		
TCT	GAA	CTT	CTA	GCC	GAT	CCG	ACC	ACT	ACA	TTT	AGC	ACA	TGG	TAT	CAC	1776
Ser	Glu	Leu	Leu	Ala	Asp	Pro	Thr	Thr	Thr	Phe	Ser	Thr	Trp	Tyr	His	
			580					585					590			
CTA	CAG	AAT	GAC	TAC	TCT	GAT	GCG	AAA	ACT	ATT	GTA	GAT	ACA	GAG	ACT	1824
Leu	Gln	Asn	Asp	Tyr	Ser	Asp	Ala	Lys	Thr	Ile	Val	Asp	Thr	Glu	Thr	
		595					600					605				

FIG. 4C

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GAA Glu	GAA Glu	AAA Lys	TCT Ser	ATA Ile	CAC His	ACT Thr	GTG Val	GAA Glu	AGT Ser	TTT Phe	AAC Asn	TCT Ser	CAA Gln	TTG Leu	GAA Glu	1872
610						615					620					
ACA Thr	CCA Pro	AAA Lys	CTT Leu	GGA Gly	TCA Ser	TGC Cys	TTA Leu	AGT Ser	AAT Asn	CTG Leu	GGA Gly	TAT Tyr	GAT Asp	GAG Glu	ACA Thr	1920
625					630					635					640	
GAT Asp	CAG Gln	TTG Leu	TCC Ser	TTT Phe	TAC Tyr	GAA Glu	GCA Ala	ATC Ile	TAT Tyr	CAA Gln	AAA Lys	AGA Arg	TCG Ser	AAC Asn	GTT Val	1968
				645					650					655		
AGA Arg	ACA Thr	AGA Arg	AGG Arg	GTT Val	AAA Lys	GTT Val	GAA Glu	GAG Glu	GAA Glu	AAT Asn	ATT Ile	GGG Gly	TAT Tyr	GCA Ala	CTA Leu	2016
			660					665					670			
AAA Lys	CAA Gln	CAA Gln	AAG Lys	AAC Asn	ACC Thr	GAA Glu	AGT Ser	TCA Ser	ACA Thr	GGG Gly	CCA Pro	CAA Gln	CTT Leu	CTG Leu	AGC Ser	2064
		675					680					685				
ATT Ile	ATT Ile	CAG Gln	ATT Ile	ATC Ile	AAA Lys	AGA Arg	ATG Met	ATT Ile	AAA Lys	AGC Ser	ATA Ile	AGA Arg	TAC Tyr	AAA Lys	AAA Lys	2112
		690				695					700					
ATT Ile	CTA Leu	ATC Ile	TTG Leu	GGA Gly	CTG Leu	CTA Leu	TGT Cys	TCT Ser	CTT Leu	ATC Ile	GCA Ala	GGC Gly	GCC Ala	ACA Thr	AAT Asn	2160
705					710					715					720	
CCC Pro	GTC Val	TTT Phe	TCA Ser	TAC Tyr	ACA Thr	TTC Phe	AGT Ser	TTC Phe	TTA Leu	CTA Leu	GAA Glu	GGA Gly	ATT Ile	GTC Val	CCA Pro	2208
				725					730					735		
TCC Ser	ACG Thr	GAT Asp	GGA Gly	AAA Lys	ACT Thr	GGC Gly	TCT Ser	TCA Ser	CAT His	TAT Tyr	TTG Leu	GCG Ala	AAA Lys	TGG Trp	TCG Ser	2256
			740					745					750			
CTT Leu	CTT Leu	GTT Val	CTT Leu	GGT Gly	GTG Val	GCT Ala	GCG Ala	GCA Ala	GAT Asp	GGT Gly	ATT Ile	TTC Phe	AAT Asn	TTT Phe	GCT Ala	2304
		755				760						765				
AAA Lys	GGA Gly	TTC Phe	CTA Leu	TTA Leu	GAT Asp	TGC Cys	TGC Cys	AGT Ser	GAA Glu	TAC Tyr	TGG Trp	GTT Val	ATG Met	GAT Asp	CTT Leu	2352
	770					775					780					
AGA Arg	AAT Asn	GAA Glu	GTT Val	ATG Met	GAA Glu	AAA Lys	CTG Leu	ACG Thr	AGA Arg	AAG Lys	AAT Asn	ATG Met	GAC Asp	TGG Trp	TTT Phe	2400
785					790					795					800	
TCT Ser	GGT Gly	GAA Glu	AAC Asn	AAC Asn	AAG Lys	GCT Ala	TCT Ser	GAA Glu	ATT Ile	TCT Ser	GCT Ala	CTA Leu	GTC Val	TTG Leu	AAT Asn	2448
				805					810					815		

FIG. 4D



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GAT	TTG	CGA	GAT	TTG	AGG	TCT	TTG	GTC	TCT	GAA	TTT	TTG	AGT	GCA	ATG	2496
Asp	Leu	Arg	Asp	Leu	Arg	Ser	Leu	Val	Ser	Glu	Phe	Leu	Ser	Ala	Met	
			820					825					830			
ACT	AGT	TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	2544
Thr	Ser	Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	
		835						840				845				
TCG	GGC	TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	2592
Ser	Gly	Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	
	850						855				860					
ATT	ATA	TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	2640
Ile	Ile	Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	
865					870					875					880	
GAT	TAT	AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	2688
Asp	Tyr	Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	
				885					890					895		
GTC	ACT	AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	2736
Val	Thr	Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	
			900					905					910			
CAA	TTG	ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	2784
Gln	Leu	Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	
		915					920					925				
AAA	CGC	GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	2832
Lys	Arg	Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	
	930						935				940					
GTC	ATG	TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	2880
Val	Met	Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr	Tyr	Gly	Leu	Lys	Leu	Val	
945					950					955					960	
ATG	ATT	CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	2928
Met	Ile	His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	
				965					970					975		
TTA	TTA	TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	2976
Leu	Leu	Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	
			980					985					990			
GAT	ATA	AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	3024
Asp	Ile	Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	
		995					1000					1005				
GAT	GAA	AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	3072
Asp	Glu	Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	
	1010						1015				1020					

FIG. 4E

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GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile 1025 1030 1035 1040	3120
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala 1045 1050 1055	3168
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu 1060 1065 1070	3216
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu 1075 1080 1085	3264
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly 1090 1095 1100	3312
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser 1105 1110 1115 1120	3360
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn 1125 1130 1135	3408
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp 1140 1145 1150	3456
GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln 1155 1160 1165	3504
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala 1170 1175 1180	3552
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu 1185 1190 1195 1200	3600
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile 1205 1210 1215	3648
ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val 1220 1225 1230	3696

FIG. 4F

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ATA	ACG	CAT	AGT	GAA	CAA	ATG	ATG	AGG	TCT	TGT	AAC	TCG	ATT	GCA	GTT	3744
Ile	Thr	His	Ser	Glu	Gln	Met	Met	Arg	Ser	Cys	Asn	Ser	Ile	Ala	Val	
		1235						1240					1245			
CTT	AAA	GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	3792
Leu	Lys	Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	
	1250						1255					1260				
AAT	AAT	CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA	3840
Asn	Asn	Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*	
1265						1270				1275					1280	

*FIG. 4G*

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## H5 Chimera (Seq. ID No. 3)

ATG	AAC	TTT	TTA	AGT	TTT	AAG	ACT	ACA	AAA	CAC	TAT	CAC	ATT	TTC	AGG	48
Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg	
1				5					10					15		
TAC	GTG	AAC	ATA	CGG	AAT	GAC	TAC	AGG	CTG	TTA	ATG	ATA	ATG	ATA	ATA	96
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile	
			20					25					30			
GGT	ACC	GTG	GCA	ACA	GGC	CTA	GTG	CCG	GCA	ATT	ACT	TCT	ATC	CTG	ACG	144
Gly	Thr	Val	Ala	Thr	Gly	Leu	Val	Pro	Ala	Ile	Thr	Ser	Ile	Leu	Thr	
		35				40						45				
GGC	AGA	GTG	TTC	GAT	CTA	CTA	TCA	GTT	TTC	GTG	GCT	AAT	GGG	TCA	CAT	192
Gly	Arg	Val	Phe	Asp	Leu	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His	
	50					55					60					
CAA	GGT	TTG	TAT	TCC	CAA	CTA	GTA	CAG	AGG	TCA	ATG	GCA	GTA	ATG	GCA	240
Gln	Gly	Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala	
65					70				75						80	
CTT	GGT	GCG	GCT	TCT	GTG	CCA	GTA	ATG	TGG	CTT	TCT	CTA	ACA	AGT	TGG	288
Leu	Gly	Ala	Ala	Ser	Val	Pro	Val	Met	Trp	Leu	Ser	Leu	Thr	Ser	Trp	
				85					90					95		
ATG	CAC	ATC	GGC	GAG	AGA	CAA	GGC	TTT	AGA	ATA	CGG	TCA	CAG	ATA	TTG	336
Met	His	Ile	Gly	Glu	Arg	Gln	Gly	Phe	Arg	Ile	Arg	Ser	Gln	Ile	Leu	
			100					105					110			
GAG	GCA	TAT	TTG	GAG	GAA	AAG	CCA	ATG	GAA	TGG	TAC	GAC	AAT	AAT	GAA	384
Glu	Ala	Tyr	Leu	Glu	Glu	Lys	Pro	Met	Glu	Trp	Tyr	Asp	Asn	Asn	Glu	
		115					120					125				
AAA	TTG	TTA	GGA	GAT	TTT	ACT	CAA	ATC	AAC	AGA	TGT	GTG	GAA	GAG	CTA	432
Lys	Leu	Leu	Gly	Asp	Phe	Thr	Gln	Ile	Asn	Arg	Cys	Val	Glu	Glu	Leu	
	130					135					140					
AGA	TCA	AGC	TCC	GCA	GAG	GCA	TCA	GCC	ATA	ACT	TTC	CAG	AAT	TTA	GTT	480
Arg	Ser	Ser	Ser	Ala	Glu	Ala	Ser	Ala	Ile	Thr	Phe	Gln	Asn	Leu	Val	
	145				150					155					160	
GCA	ATA	TGT	GCG	CTT	CTG	GGG	ACG	TCA	TTC	TAC	TAT	TCT	TGG	TCA	TTA	528
Ala	Ile	Cys	Ala	Leu	Leu	Gly	Thr	Ser	Phe	Tyr	Tyr	Ser	Trp	Ser	Leu	
			165						170					175		
ACT	TTA	ATT	ATT	CTT	TGC	AGC	TCT	CCA	ATA	ATC	ACA	TTT	TTT	GCA	GTG	576
Thr	Leu	Ile	Ile	Leu	Cys	Ser	Ser	Pro	Ile	Ile	Thr	Phe	Phe	Ala	Val	
			180					185					190			

FIG. 5A

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GTG	TTT	TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	624
Val	Phe	Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	
		195					200					205				
ACG	AGT	AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	672
Thr	Ser	Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	
	210					215					220					
TTA	GTG	AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	720
Leu	Val	Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	
	225				230					235					240	
GAA	ATC	ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTT	ATC	AAG	AGT	TGC	TTT	TTT	768
Glu	Ile	Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	
				245					250					255		
GTT	GCT	GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	816
Val	Ala	Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	
			260					265					270			
GTA	CAG	GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	864
Val	Gln	Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	
		275					280					285				
AAC	ATT	AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	912
Asn	Ile	Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	
	290					295					300					
TCG	ACT	TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	960
Ser	Thr	Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	
	305				310					315					320	
GGA	GTG	GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	1008
Gly	Val	Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	
				325					330					335		
CGA	AAT	CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	1056
Arg	Asn	Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	
			340					345					350			
GCC	ACC	AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	1104
Ala	Thr	Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	
		355				360						365				
AGA	CCT	TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	1152
Arg	Pro	Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	
	370					375					380					
GGA	CAA	TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	1200
Gly	Gln	Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	
	385				390					395					400	

FIG. 5B

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TTA TCC AAC TTA TTA TTA AGG TTC TAC GAT GGC TAT AAT GGA TCG ATA	1248
Leu Ser Asn Leu Leu Leu Arg Phe Tyr Asp Gly Tyr Asn Gly Ser Ile	
405 410 415	
TCT ATC AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT	1296
Ser Ile Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile	
420 425 430	
GAA AAT ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC	1344
Glu Asn Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr	
435 440 445	
STE6 CFTR	
ATT AAA GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC	1392
Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr	
450 455 460	
AGA AGC GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT	1440
Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe	
465 470 475 480	
GCA GAG AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT	1488
Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser	
485 490 495	
GGA GGT CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA TTC ATC AGA GAT	1536
Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Phe Ile Arg Asp	
500 505 510	
CFTR STE6	
ACT CCA ATA TTA TTC TTA GAC GAA GCT GTA TCG GCT CTA GAT ATT GTT	1584
Thr Pro Ile Leu Phe Leu Asp Glu Ala Val Ser Ala Leu Asp Ile Val	
515 520 525	
CAT CGC AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG	1632
His Arg Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys	
530 535 540	
ACT ACA ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC	1680
Thr Thr Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp	
545 550 555 560	
TAT TTA TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG	1728
Tyr Leu Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln	
565 570 575	
TCT GAA CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC	1776
Ser Glu Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His	
580 585 590	
CTA CAG AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT	1824
Leu Gln Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr	
595 600 605	

FIG. 5C

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GAA GAA AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA Glu Glu Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu 610 615 620	1872
ACA CCA AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA Thr Pro Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr 625 630 635 640	1920
GAT CAG TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT Asp Gln Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val 645 650 655	1968
AGA ACA AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA Arg Thr Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu 660 665 670	2016
AAA CAA CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC Lys Gln Gln Lys Asn Thr Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser 675 680 685	2064
ATT ATT CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA Ile Ile Gln Ile Ile Lys Arg Met Ile Lys Ser Ile Arg Tyr Lys Lys 690 695 700	2112
ATT CTA ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT Ile Leu Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn 705 710 715 720	2160
CCC GTC TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA Pro Val Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro 725 730 735	2208
TCC ACG GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG Ser Thr Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser 740 745 750	2256
CTT CTT GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT Leu Leu Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala 755 760 765	2304
AAA GGA TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT Lys Gly Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu 770 775 780	2352
AGA AAT GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT Arg Asn Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe 785 790 795 800	2400
TCT GGT GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT Ser Gly Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn 805 810 815	2448

FIG. 5D

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GAT	TTG	CGA	GAT	TTG	AGG	TCT	TTG	GTC	TCT	GAA	TTT	TTG	AGT	GCA	ATG	2496
Asp	Leu	Arg	Asp	Leu	Arg	Ser	Leu	Val	Ser	Glu	Phe	Leu	Ser	Ala	Met	
			820					825					830			
ACT	AGT	TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	2544
Thr	Ser	Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	
		835					840					845				
TCG	GGC	TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	2592
Ser	Gly	Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	
	850					855					860					
ATT	ATA	TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	2640
Ile	Ile	Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	
865					870					875					880	
GAT	TAT	AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	2688
Asp	Tyr	Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	
				885					890					895		
GTC	ACT	AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	2736
Val	Thr	Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	
			900					905					910			
CAA	TTG	ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	2784
Gln	Leu	Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	
		915					920					925				
AAA	CGC	GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	2832
Lys	Arg	Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	
	930					935					940					
GTC	ATG	TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	2880
Val	Met	Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr		Gly	Leu	Lys	Leu	Val	
945					950					955					960	
ATG	ATT	CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	2928
Met	Ile	His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	
				965					970					975		
TTA	TTA	TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	2976
Leu	Leu	Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	
			980					985					990			
GAT	ATA	AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	3024
Asp	Ile	Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	
		995					1000					1005				
GAT	GAA	AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	3072
Asp	Glu	Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	
	1010					1015					1020					

FIG.5E



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GTG GGA ATA GCT GGT CAC ACC TAC CAT GGC AAA GAA AAA AAA CCA ATC	3120
Val Gly Ile Ala Gly His Thr Tyr His Gly Lys Glu Lys Lys Pro Ile	
1025 1030 1035 1040	
GTT TCA ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC	3168
Val Ser Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala	
1045 1050 1055	
TTT GTT TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA	3216
Phe Val Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu	
1060 1065 1070	
GGT ATC ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA	3264
Gly Ile Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu	
1075 1080 1085	
TTA ACA AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT	3312
Leu Thr Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly	
1090 1095 1100	
ACG GAC GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA	3360
Thr Asp Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser	
1105 1110 1115 1120	
GTG GTT GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC	3408
Val Val Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn	
1125 1130 1135	
CTA ACT TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT	3456
Leu Thr Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp	
1140 1145 1150	
GCA TTA AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG	3504
Ala Leu Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln	
1155 1160 1165	
GGC TTG GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG	3552
Gly Leu Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala	
1170 1175 1180	
CAA AGG CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG	3600
Gln Arg Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu	
1185 1190 1195 1200	
ATT TTA GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC	3648
Ile Leu Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile	
1205 1210 1215	
ATC AAT GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT	3696
Ile Asn Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val	
1220 1225 1230	

FIG. 5F

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ATA	ACG	CAT	AGT	GAA	CAA	ATG	ATG	AGG	TCT	TGT	AAC	TCG	ATT	GCA	GTT	3744
Ile	Thr	His	Ser	Glu	Gln	Met	Met	Arg	Ser	Cys	Asn	Ser	Ile	Ala	Val	
		1235						1240						1245		
CTT	AAA	GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	3792
Leu	Lys	Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	
		1250						1255						1260		
AAT	AAT	CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA	3840
Asn	Asn	Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*	
		1265						1270						1275		1280

*FIG.5G*

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## H6 Chimera (Seq. ID No. 5)

ATG	AAC	TTT	TTA	AGT	TTT	AAG	ACT	ACA	AAA	CAC	TAT	CAC	ATT	TTC	AGG	48
Met	Asn	Phe	Leu	Ser	Phe	Lys	Thr	Thr	Lys	His	Tyr	His	Ile	Phe	Arg	
1				5					10					15		
TAC	GTG	AAC	ATA	CGG	AAT	GAC	TAC	AGG	CTG	TTA	ATG	ATA	ATG	ATA	ATA	96
Tyr	Val	Asn	Ile	Arg	Asn	Asp	Tyr	Arg	Leu	Leu	Met	Ile	Met	Ile	Ile	
			20					25					30			
GGT	ACC	GTG	GCA	CTA	TCA	GTT	TTC	GTG	GCT	AAT	GGG	TCA	CAT	CAA	GGT	144
Gly	Thr	Val	Ala	Leu	Ser	Val	Phe	Val	Ala	Asn	Gly	Ser	His	Gln	Gly	
		35					40					45				
TTG	TAT	TCC	CAA	CTA	GTA	CAG	AGG	TCA	ATG	GCA	GTA	ATG	GCA	CTT	GGT	192
Leu	Tyr	Ser	Gln	Leu	Val	Gln	Arg	Ser	Met	Ala	Val	Met	Ala	Leu	Gly	
	50					55					60					
GCG	GCT	TCT	GTG	CCA	GTA	ATG	TGG	CTT	TCT	CTA	ACA	AGT	TGG	ATG	CAC	240
Ala	Ala	Ser	Val	Pro	Val	Met	Trp	Leu	Ser	Leu	Thr	Ser	Trp	Met	His	
65					70					75					80	
ATC	GGC	GAG	AGA	CAA	GGC	TTT	AGA	ATA	CGG	TCA	CAG	ATA	TTG	GAG	GCA	288
Ile	Gly	Glu	Arg	Gln	Gly	Phe	Arg	Ile	Arg	Ser	Gln	Ile	Leu	Glu	Ala	
				85					90					95		
TAT	TTG	GAG	GAA	AAG	CCA	ATG	GAA	TGG	TAC	GAC	AAT	AAT	GAA	AAA	TTG	336
Tyr	Leu	Glu	Glu	Lys	Pro	Met	Glu	Trp	Tyr	Asp	Asn	Asn	Glu	Lys	Leu	
			100					105					110			
TTA	GGA	GAT	TTT	ACT	CAA	ATC	AAC	AGA	TGT	GTG	GAA	GAG	CTA	AGA	TCA	384
Leu	Gly	Asp	Phe	Thr	Gln	Ile	Asn	Arg	Cys	Val	Glu	Glu	Leu	Arg	Ser	
		115					120					125				
AGC	TCC	GCA	GAG	GCA	TCA	GCC	ATA	ACT	TTC	CAG	AAT	TTA	GTT	GCA	ATA	432
Ser	Ser	Ala	Glu	Ala	Ser	Ala	Ile	Thr	Phe	Gln	Asn	Leu	Val	Ala	Ile	
		130				135					140					
TGT	GCG	CTT	CTG	GGG	ACG	TCA	TTC	TAC	TAT	TCT	TGG	TCA	TTA	ACT	TTA	480
Cys	Ala	Leu	Leu	Gly	Thr	Ser	Phe	Tyr	Tyr	Ser	Trp	Ser	Leu	Thr	Leu	
145					150					155					160	
ATT	ATT	CTT	TGC	AGC	TCT	CCA	ATA	ATC	ACA	TTT	TTT	GCA	GTG	GTG	TTT	528
Ile	Ile	Leu	Cys	Ser	Ser	Pro	Ile	Ile	Thr	Phe	Phe	Ala	Val	Val	Phe	
				165					170					175		
TCC	AGA	ATG	ATT	CAT	GTA	TAT	TCA	GAG	AAG	GAG	AAT	TCT	GAA	ACG	AGT	576
Ser	Arg	Met	Ile	His	Val	Tyr	Ser	Glu	Lys	Glu	Asn	Ser	Glu	Thr	Ser	
			180					185					190			

FIG. 6A

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AAA	GCA	GCC	CAA	TTA	CTT	ACA	TGG	TCG	ATG	AAT	GCC	GCT	CAA	TTA	GTG	624
Lys	Ala	Ala	Gln	Leu	Leu	Thr	Trp	Ser	Met	Asn	Ala	Ala	Gln	Leu	Val	
		195					200					205				
AGA	TTA	TAT	TGT	ACA	CAA	CGT	CTA	GAA	AGG	AAA	AAA	TTC	AAG	GAA	ATC	672
Arg	Leu	Tyr	Cys	Thr	Gln	Arg	Leu	Glu	Arg	Lys	Lys	Phe	Lys	Glu	Ile	
	210					215					220					
ATA	CTA	AAT	TGT	AAC	ACT	TTC	TTC	ATC	AAG	AGT	TGC	TTT	TTT	GTT	GCT	720
Ile	Leu	Asn	Cys	Asn	Thr	Phe	Phe	Ile	Lys	Ser	Cys	Phe	Phe	Val	Ala	
225					230					235					240	
GCA	AAC	GCT	GGG	ATC	TTG	AGA	TTT	TTG	ACG	TTG	ACT	ATG	TTT	GTA	CAG	768
Ala	Asn	Ala	Gly	Ile	Leu	Arg	Phe	Leu	Thr	Leu	Thr	Met	Phe	Val	Gln	
			245						250					255		
GGA	TTC	TGG	TTT	GGT	TCC	GCA	ATG	ATC	AAA	AAG	GGC	AAG	CTG	AAC	ATT	816
Gly	Phe	Trp	Phe	Gly	Ser	Ala	Met	Ile	Lys	Lys	Gly	Lys	Leu	Asn	Ile	
		260						265					270			
AAC	GAT	GTA	ATC	ACT	TGC	TTC	CAT	TCA	TGC	ATT	ATG	CTG	GGC	TCG	ACT	864
Asn	Asp	Val	Ile	Thr	Cys	Phe	His	Ser	Cys	Ile	Met	Leu	Gly	Ser	Thr	
		275					280					285				
TTA	AAT	AAT	ACA	TTA	CAC	CAA	ATA	GTT	GTT	CTT	CAA	AAA	GGC	GGA	GTG	912
Leu	Asn	Asn	Thr	Leu	His	Gln	Ile	Val	Val	Leu	Gln	Lys	Gly	Gly	Val	
	290					295					300					
GCT	ATG	GAA	AAA	ATC	ATG	ACT	CTA	TTA	AAA	GAT	GGA	TCC	AAG	CGA	AAT	960
Ala	Met	Glu	Lys	Ile	Met	Thr	Leu	Leu	Lys	Asp	Gly	Ser	Lys	Arg	Asn	
305					310					315					320	
CCT	TTA	AAT	AAA	ACT	GTA	GCC	CAC	CAA	TTT	CCA	CTA	GAT	TAT	GCC	ACC	1008
Pro	Leu	Asn	Lys	Thr	Val	Ala	His	Gln	Phe	Pro	Leu	Asp	Tyr	Ala	Thr	
			325						330					335		
AGT	GAT	CTA	ACA	TTT	GCT	AAT	GTT	TCG	TTT	TCT	TAT	CCA	AGC	AGA	CCT	1056
Ser	Asp	Leu	Thr	Phe	Ala	Asn	Val	Ser	Phe	Ser	Tyr	Pro	Ser	Arg	Pro	
		340						345					350			
TCG	GAA	GCA	GTT	TTA	AAG	AAC	GTT	AGT	TTA	AAT	TTC	TCT	GCA	GGA	CAA	1104
Ser	Glu	Ala	Val	Leu	Lys	Asn	Val	Ser	Leu	Asn	Phe	Ser	Ala	Gly	Gln	
		355					360					365				
TTT	ACT	TTC	ATA	GTA	GGA	AAA	TCA	GGC	TCA	GGT	AAA	TCT	ACA	TTA	TCC	1152
Phe	Thr	Phe	Ile	Val	Gly	Lys	Ser	Gly	Ser	Gly	Lys	Ser	Thr	Leu	Ser	
	370					375					380					
AAC	TTA	TTA	TTA	AGG	TTC	TAC	GAT	GGC	TAT	AAT	GGA	TCG	ATA	TCT	ATC	1200
Asn	Leu	Leu	Leu	Arg	Phe	Tyr	Asp	Gly	Tyr	Asn	Gly	Ser	Ile	Ser	Ile	
385					390					395					400	

FIG. 6B

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AAT GGC CAC AAT ATC CAA ACA ATC GAC CAA AAA TTG CTA ATT GAA AAT	1248
Asn Gly His Asn Ile Gln Thr Ile Asp Gln Lys Leu Leu Ile Glu Asn	
405 410 415	
ATC ACC GTC GTA GAA CAG TTT TCC TGG ATT ATG CCT GGC ACC ATT AAA	1296
Ile Thr Val Val Glu Gln Phe Ser Trp Ile Met Pro Gly Thr Ile Lys	
420 425 430	
STE6 CFTR	
GAA AAT ATC ATC TTT GGT GTT TCC TAT GAT GAA TAT AGA TAC AGA AGC	1344
Glu Asn Ile Ile Phe Gly Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser	
435 440 445	
GTC ATC AAA GCA TGC CAA CTA GAA GAG GAC ATC TCC AAG TTT GCA GAG	1392
Val Ile Lys Ala Cys Gln Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu	
450 455 460	
AAA GAC AAT ATA GTT CTT GGA GAA GGT GGA ATC ACA CTG AGT GGA GGT	1440
Lys Asp Asn Ile Val Leu Gly Glu Gly Gly Ile Thr Leu Ser Gly Gly	
465 470 475 480	
CAA CGA GCA AGA ATT TCT TTA GCA AGA GCA GTA TAC AAA GAT GCT GAT	1488
Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp	
485 490 495	
TTG TAT TTA TTA GAC TCT CCT TTT GGA TAC CTA GAT ATT GTT CAT CGC	1536
Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr Leu Asp Ile Val His Arg	
500 505 510	
CFTR STE6	
AAC CTG TTG ATG AAG GCA ATT AGG CAT TGG AGG AAA GGA AAG ACT ACA	1584
Asn Leu Leu Met Lys Ala Ile Arg His Trp Arg Lys Gly Lys Thr Thr	
515 520 525	
ATC ATA TTG ACG CAT GAG TTG AGC CAA ATT GAA TCT GAT GAC TAT TTA	1632
Ile Ile Leu Thr His Glu Leu Ser Gln Ile Glu Ser Asp Asp Tyr Leu	
530 535 540	
TAT TTA ATG AAG GAA GGT GAA GTT GTT GAA AGC GGC ACC CAG TCT GAA	1680
Tyr Leu Met Lys Glu Gly Glu Val Val Glu Ser Gly Thr Gln Ser Glu	
545 550 555 560	
CTT CTA GCC GAT CCG ACC ACT ACA TTT AGC ACA TGG TAT CAC CTA CAG	1728
Leu Leu Ala Asp Pro Thr Thr Thr Phe Ser Thr Trp Tyr His Leu Gln	
565 570 575	
AAT GAC TAC TCT GAT GCG AAA ACT ATT GTA GAT ACA GAG ACT GAA GAA	1776
Asn Asp Tyr Ser Asp Ala Lys Thr Ile Val Asp Thr Glu Thr Glu Glu	
580 585 590	
AAA TCT ATA CAC ACT GTG GAA AGT TTT AAC TCT CAA TTG GAA ACA CCA	1824
Lys Ser Ile His Thr Val Glu Ser Phe Asn Ser Gln Leu Glu Thr Pro	
595 600 605	

FIG. 6C

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AAA CTT GGA TCA TGC TTA AGT AAT CTG GGA TAT GAT GAG ACA GAT CAG Lys Leu Gly Ser Cys Leu Ser Asn Leu Gly Tyr Asp Glu Thr Asp Gln 610 615 620	1872
TTG TCC TTT TAC GAA GCA ATC TAT CAA AAA AGA TCG AAC GTT AGA ACA Leu Ser Phe Tyr Glu Ala Ile Tyr Gln Lys Arg Ser Asn Val Arg Thr 625 630 635 640	1920
AGA AGG GTT AAA GTT GAA GAG GAA AAT ATT GGG TAT GCA CTA AAA CAA Arg Arg Val Lys Val Glu Glu Glu Asn Ile Gly Tyr Ala Leu Lys Gln 645 650 655	1968
CAA AAG AAC ACC GAA AGT TCA ACA GGG CCA CAA CTT CTG AGC ATT ATT Gln Lys Asn Glu Ser Ser Thr Gly Pro Gln Leu Leu Ser Ile Ile 660 665 670	2016
CAG ATT ATC AAA AGA ATG ATT AAA AGC ATA AGA TAC AAA AAA ATT CTA Gln Ile Ile Lys Arg Met Ile Ser Ile Arg Tyr Lys Lys Ile Leu 675 680 685	2064
ATC TTG GGA CTG CTA TGT TCT CTT ATC GCA GGC GCC ACA AAT CCC GTC Ile Leu Gly Leu Leu Cys Ser Leu Ile Ala Gly Ala Thr Asn Pro Val 690 695 700	2112
TTT TCA TAC ACA TTC AGT TTC TTA CTA GAA GGA ATT GTC CCA TCC ACG Phe Ser Tyr Thr Phe Ser Phe Leu Leu Glu Gly Ile Val Pro Ser Thr 705 710 715 720	2160
GAT GGA AAA ACT GGC TCT TCA CAT TAT TTG GCG AAA TGG TCG CTT CTT Asp Gly Lys Thr Gly Ser Ser His Tyr Leu Ala Lys Trp Ser Leu Leu 725 730 735	2208
GTT CTT GGT GTG GCT GCG GCA GAT GGT ATT TTC AAT TTT GCT AAA GGA Val Leu Gly Val Ala Ala Ala Asp Gly Ile Phe Asn Phe Ala Lys Gly 740 745 750	2256
TTC CTA TTA GAT TGC TGC AGT GAA TAC TGG GTT ATG GAT CTT AGA AAT Phe Leu Leu Asp Cys Cys Ser Glu Tyr Trp Val Met Asp Leu Arg Asn 755 760 765	2304
GAA GTT ATG GAA AAA CTG ACG AGA AAG AAT ATG GAC TGG TTT TCT GGT Glu Val Met Glu Lys Leu Thr Arg Lys Asn Met Asp Trp Phe Ser Gly 770 775 780	2352
GAA AAC AAC AAG GCT TCT GAA ATT TCT GCT CTA GTC TTG AAT GAT TTG Glu Asn Asn Lys Ala Ser Glu Ile Ser Ala Leu Val Leu Asn Asp Leu 785 790 795 800	2400
CGA GAT TTG AGG TCT TTG GTC TCT GAA TTT TTG AGT GCA ATG ACT AGT Arg Asp Leu Arg Ser Leu Val Ser Glu Phe Leu Ser Ala Met Thr Ser 805 810 815	2448

FIG. 6D

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TTC	GTT	ACC	GTA	TCA	ACG	ATT	GGA	CTA	ATT	TGG	GCG	TTA	GTA	TCG	GGC	2496
Phe	Val	Thr	Val	Ser	Thr	Ile	Gly	Leu	Ile	Trp	Ala	Leu	Val	Ser	Gly	
			820					825					830			
TGG	AAG	TTA	AGT	TTG	GTT	TGT	ATT	TCG	ATG	TTT	CCA	CTC	ATA	ATT	ATA	2544
Trp	Lys	Leu	Ser	Leu	Val	Cys	Ile	Ser	Met	Phe	Pro	Leu	Ile	Ile	Ile	
		835					840					845				
TTT	TCA	GCA	ATA	TAT	GGA	GGC	ATT	TTA	CAA	AAA	TGC	GAA	ACA	GAT	TAT	2592
Phe	Ser	Ala	Ile	Tyr	Gly	Gly	Ile	Leu	Gln	Lys	Cys	Glu	Thr	Asp	Tyr	
	850					855					860					
AAG	ACA	TCT	GTT	GCT	CAG	TTA	GAA	AAC	TGC	CTG	TAC	CAG	ATT	GTC	ACT	2640
Lys	Thr	Ser	Val	Ala	Gln	Leu	Glu	Asn	Cys	Leu	Tyr	Gln	Ile	Val	Thr	
865					870					875					880	
AAC	ATT	AAA	ACC	ATT	AAG	TGC	TTA	CAA	GCT	GAA	TTT	CAT	TTT	CAA	TTG	2688
Asn	Ile	Lys	Thr	Ile	Lys	Cys	Leu	Gln	Ala	Glu	Phe	His	Phe	Gln	Leu	
				885				890						895		
ACC	TAC	CAT	GAC	TTG	AAG	ATA	AAA	ATG	CAA	CAA	ATT	GCC	TCC	AAA	CGC	2736
Thr	Tyr	His	Asp	Leu	Lys	Ile	Lys	Met	Gln	Gln	Ile	Ala	Ser	Lys	Arg	
			900					905					910			
GCC	ATT	GCC	ACA	GGA	TTT	GGT	ATA	TCT	ATG	ACA	AAC	ATG	ATT	GTC	ATG	2784
Ala	Ile	Ala	Thr	Gly	Phe	Gly	Ile	Ser	Met	Thr	Asn	Met	Ile	Val	Met	
		915					920					925				
TGT	ATC	CAA	GCT	ATT	ATT	TAC	TAC	TAT	GGC	CTA	AAG	CTG	GTT	ATG	ATT	2832
Cys	Ile	Gln	Ala	Ile	Ile	Tyr	Tyr	Tyr	Gly	Leu	Lys	Leu	Val	Met	Ile	
	930					935					940					
CAC	GAG	TAC	ACC	TCA	AAG	GAA	ATG	TTT	ACG	ACT	TTC	ACT	TTG	TTA	TTA	2880
His	Glu	Tyr	Thr	Ser	Lys	Glu	Met	Phe	Thr	Thr	Phe	Thr	Leu	Leu	Leu	
945					950					955					960	
TTC	ACT	ATT	ATG	TCA	TGC	ACT	AGC	CTA	GTA	AGT	CAG	ATA	CCC	GAT	ATA	2928
Phe	Thr	Ile	Met	Ser	Cys	Thr	Ser	Leu	Val	Ser	Gln	Ile	Pro	Asp	Ile	
				965				970						975		
AGT	AGA	GGC	CAA	CGT	GCT	GCC	AGT	TGG	ATC	TAT	AGG	ATT	CTT	GAT	GAA	2976
Ser	Arg	Gly	Gln	Arg	Ala	Ala	Ser	Trp	Ile	Tyr	Arg	Ile	Leu	Asp	Glu	
		980						985					990			
AAG	CAT	AAT	ACC	CTA	GAG	GTT	GAA	AAC	AAT	AAT	GCT	AGA	ACA	GTG	GGA	3024
Lys	His	Asn	Thr	Leu	Glu	Val	Glu	Asn	Asn	Asn	Ala	Arg	Thr	Val	Gly	
		995					1000					1005				
ATA	GCT	GGT	CAC	ACC	TAC	CAT	GGC	AAA	GAA	AAA	AAA	CCA	ATC	GTT	TCA	3072
Ile	Ala	Gly	His	Thr	Tyr	His	Gly	Lys	Glu	Lys	Lys	Pro	Ile	Val	Ser	
	1010						1015					1020				

FIG. 6E

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ATT CAA AAT TTG ACA TTT GCC TAT CCA TCT GCA CCT ACC GCC TTT GTT Ile Gln Asn Leu Thr Phe Ala Tyr Pro Ser Ala Pro Thr Ala Phe Val 1025 1030 1035 1040	3120
TAC AAA AAC ATG AAT TTT GAC ATG TTT TGC GGA CAG ACG TTA GGT ATC Tyr Lys Asn Met Asn Phe Asp Met Phe Cys Gly Gln Thr Leu Gly Ile 1045 1050 1055	3168
ATT GGT GAA TCA GGC ACA GGA AAG TCT ACA CTT GTG CTT TTA TTA ACA Ile Gly Glu Ser Gly Thr Gly Lys Ser Thr Leu Val Leu Leu Leu Thr 1060 1065 1070	3216
AAA CTT TAT AAT TGT GAA GTA GGC AAA ATT AAA ATA GAC GGT ACG GAC Lys Leu Tyr Asn Cys Glu Val Gly Lys Ile Lys Ile Asp Gly Thr Asp 1075 1080 1085	3264
GTA AAT GAC TGG AAT TTG ACA AGT TTA AGA AAA GAA ATT TCA GTG GTT Val Asn Asp Trp Asn Leu Thr Ser Leu Arg Lys Glu Ile Ser Val Val 1090 1095 1100	3312
GAG CAA AAA CCT TTA TTA TTC AAT GGA ACC ATC AGA GAT AAC CTA ACT Glu Gln Lys Pro Leu Leu Phe Asn Gly Thr Ile Arg Asp Asn Leu Thr 1105 1110 1115 1120	3360
TAT GGT TTA CAA GAT GAA ATA CTT GAA ATT GAA ATG TAT GAT GCA TTA Tyr Gly Leu Gln Asp Glu Ile Leu Glu Ile Glu Met Tyr Asp Ala Leu 1125 1130 1135	3408
AAA TAC GTA GGA ATC CAT GAC TTT GTA ATT TCA TCA CCT CAG GGC TTG Lys Tyr Val Gly Ile His Asp Phe Val Ile Ser Ser Pro Gln Gly Leu 1140 1145 1150	3456
GAT ACA CGT ATT GAT ACA ACT TTA CTG TCA GGT GGA CAA GCG CAA AGG Asp Thr Arg Ile Asp Thr Thr Leu Leu Ser Gly Gly Gln Ala Gln Arg 1155 1160 1165	3504
CTT TGC ATA GCC AGA GCA CTT CTG AGA AAA TCA AAA ATT CTG ATT TTA Leu Cys Ile Ala Arg Ala Leu Leu Arg Lys Ser Lys Ile Leu Ile Leu 1170 1175 1180	3552
GAT GAG TGT ACT TCA GCC TTG GAT TCT GTC AGC TCC TCT ATC ATC AAT Asp Glu Cys Thr Ser Ala Leu Asp Ser Val Ser Ser Ser Ile Ile Asn 1185 1190 1195 1200	3600
GAG ATC GTC AAA AAA GGT CCA CCT GCT CTA CTA ACA ATG GTT ATA ACG Glu Ile Val Lys Lys Gly Pro Pro Ala Leu Leu Thr Met Val Ile Thr 1205 1210 1215	3648
CAT AGT GAA CAA ATG ATG AGG TCT TGT AAC TCG ATT GCA GTT CTT AAA His Ser Glu Gln Met Met Arg Ser Cys Asn Ser Ile Ala Val Leu Lys 1220 1225 1230	3696

FIG. 6F



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GAT	GGT	AAA	GTG	GTT	GAG	CGA	GGT	AAC	TTC	GAC	ACT	TTA	TAT	AAT	AAT	3744
Asp	Gly	Lys	Val	Val	Glu	Arg	Gly	Asn	Phe	Asp	Thr	Leu	Tyr	Asn	Asn	
		1235					1240					1245				
CGC	GGG	GAA	TTA	TTC	CAA	ATT	GTT	TCC	AAC	CAA	AGC	AGT	TAA			3786
Arg	Gly	Glu	Leu	Phe	Gln	Ile	Val	Ser	Asn	Gln	Ser	Ser	*			
		1250					1255					1260				

*FIG. 6G*

## INTERNATIONAL SEARCH REPORT

Internat. Application No  
PCT 94/04379

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/62 C07K13/00 G01N33/50 C12N1/19

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CELLULAR BIOCHEMISTRY Supplement 16F; April 3-16, 1992 page 15 see abstract no. U 116 ---	1,2,6-8, 13-15
A	MOLECULAR BIOLOGY OF THE CELL vol. 3, September 1992 page 192A Y. MCCLENDON ET AL. 'Analysis of cystic fibrosis-related mutations in the yeast Ste6 protein' see abstract no. 1116 --- -/--	1,15



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

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Date of the actual completion of the international search

9 September 1994

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## INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/US 94/04379

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CELL vol. 73, no. 2 , 23 April 1993 , CAMBRIDGE, MA US pages 335 - 346 JOHN L. TEEM ET AL. 'Identification of revertants for the cystic fibrosis deltaF508 mutation using STE6-CFTR chimeras in yeast' see summary see page 335, right column, paragraph 4 - page 336, right column, paragraph 2 see page 336, right column, paragraph 4 - page 338, left column, paragraph 1 see page 342, left column, paragraph 2 - right column, paragraph 1; figure 1 ---	1-15
P,X	BIOCHEMISTRY AND CELL BIOLOGY vol. 71, no. 11-2 , December 1993 page AXXV JOHN L. TEEM ET AL. 'The construction of STE6/CFTR chimeric transporters to define functional similarities between transmembrane domain segments' see abstract -----	1,7, 13-15